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Sahni, B. (1936a) Wegener's theory of continental drift in the light of Palaeobotanical evidence. *J. Indian bot. Soc.*, 15: 31-32.

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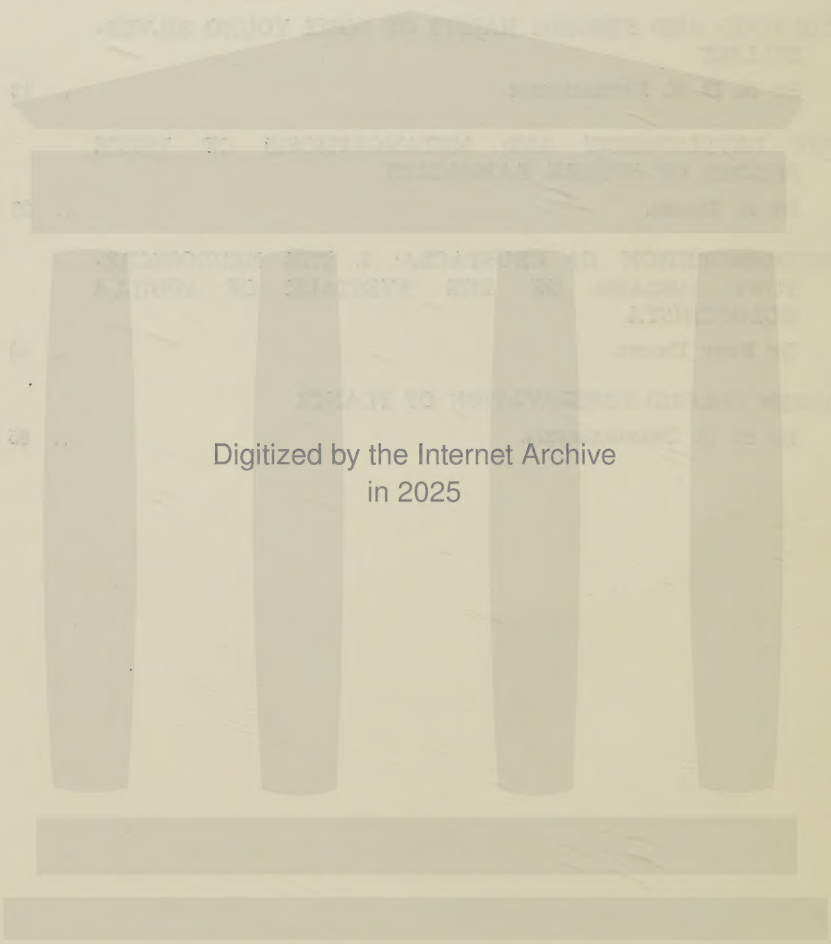

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Studies on Post Larvae and Feeding Habits of Some Fishes Found in the Madras Plankton

BY

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(Received for publication 29th October 1957)

ABSTRACT

1. In the present investigation extending over a period of one and half years from 1955 June to 1957 March, the stomach contents of 164 larvae belonging to 6 species were examined for determining their food and feeding habits of fishes. A brief resume of the previous work regarding the food and feeding habits of larval forms of fishes done both in India and abroad is given.

2. The young forms of almost all the species studied are carnivorus surface plankton feeders.

3. Decapods formed the main food item of young forms of *Elops saurus*, *Hemiramphus* sp. and Gobius sp. where as *Saurida* sp. and *Megalops cyprinoides* seem to have a partiality for copepods.

4. The younger forms of *Stolephorus* sp. and *Ambassis* sp. feed on larval and post larval forms of fishes such as *Clupea* sp., *Leiognathus* sp., *Caranx* sp., *Elops* sp., *Engraulis* sp., *Stolephorus* sp. and *Trichiurus* sp. which are of market value.

Introduction

With a view of studying the larval stages of the common fishes occurring along the coast of Madras, regular plankton collections were made from the sea and from the brackish waters of Coovum and Adyar throughout the period of investigation. The period of occurrence of these larvae, the larval characteristic features and their feeding habits were described in detail. Apart from a few descriptions of the larval and post larval forms of some of the marine and brackish water fishes of the tropics by authors like Chidambaram (1941), Gopinath (1948), Bapat and Bal (1952), Bhattacharya (1916), Sundararaj (1916), Southwell and Prashad (1919), Jones (1937), Job and Jones (1938), Rao and Hora (1938), Nair (1940 & 1952), Jones and Menon (1951), Nair (1952), John (1951), and Vijayaraghavan (1953, unpublished thesis) and (1955),

etc., no attempt has been made to study exclusively the larval and post larval forms. The work of Delsman (1921-'38) on the fish eggs and larvae collected from the Java Sea dealt with the developmental stages of the fishes of the Indo-Pacific region. But he confined himself mainly to the description of the eggs and the embryonic forms. Though the description of the eggs of some and larvae of others can be found in the papers of several authors in the sub-tropics Lebour (1918-1925) who has published a series of papers on the food and feeding habits of larval and post larval fishes of the fishery grounds off Plymouth deserves mention.

Material and Methods

All the fishes studied were collected from the Madras Plankton and brought to the laboratory every morning. The Coovum and Adyar plankton samples were made by the author himself, whereas the inshore waters of the Bay of Bengal were townetted every day by a fisherman who went out with a native craft. From the spot of collection, the plankton was carried to the laboratory in a porous mud pot and transferred to a glass trough. A total of 164 larvae were collected from the sea as well as from the brackish waters. Due to rough handling many of the delicate larvae did not survive and those live ones which were brought to the laboratory also died after some hours. The larvae were immediately transferred into 5% formalin and figured, noting the number of myotomes and other important characteristic of the species. Later on, their stomachs were examined in order to determine their feeding habits. Six different kinds of larvae were presented but specific identification of the species could not be made out due to lack of literature but wherever possible identification of the species also was made. Clark (1920) has observed, the post larvae were so different from the adult that characters well known for the identification of the latter were of little use in the identification of the former, while the development of additional characters rendered them entirely different from the embroyonic forms. During the course of the study from 1955 June to 1957 March, since no earlier or older stages were available the only post larvae obtained has been presented in this paper with a view to facilitate the study by others in this direction.

Elops saurus: (*Swaimson*) (Fig. I)

The larval stages of *Elops saurus* have been recorded by Baj (loc. cit), Panikkar and Iyer (1939), John (1944) and Nair (loc.

cit.) from Madras waters and Delsman (1926) from Java Seas. Gopinath (loc. cit.) recorded *Elops indicus* from Trivandrum Coast. The present species were obtained from the Plankton collections from the sea during the months of September and October. They were identified as *Elops saurus* from the description of the above authors.

The larvae of *Elops saurus* were transparent and measured 14·2 mm. length. About 18 larvae were collected and they were ribbon like, like the leptocephalus of eels. The head was very small when compared with the body and had a sharply pointed snout. The alimentary canal was long and straight and the anus situated under the 58th myotome. An air bladder was noticed in the 31st myotome. Along the dorsal aspect of the alimentary canal and throughout its length, black stellate chromatophores were aranged in lines and the rest of the body was free from pigmentation. The eyes were pigmented and the iris was formed. The dorsal and ventral fins were devoid of fin rays and the caudal was deeply forked with numerous fin rays.

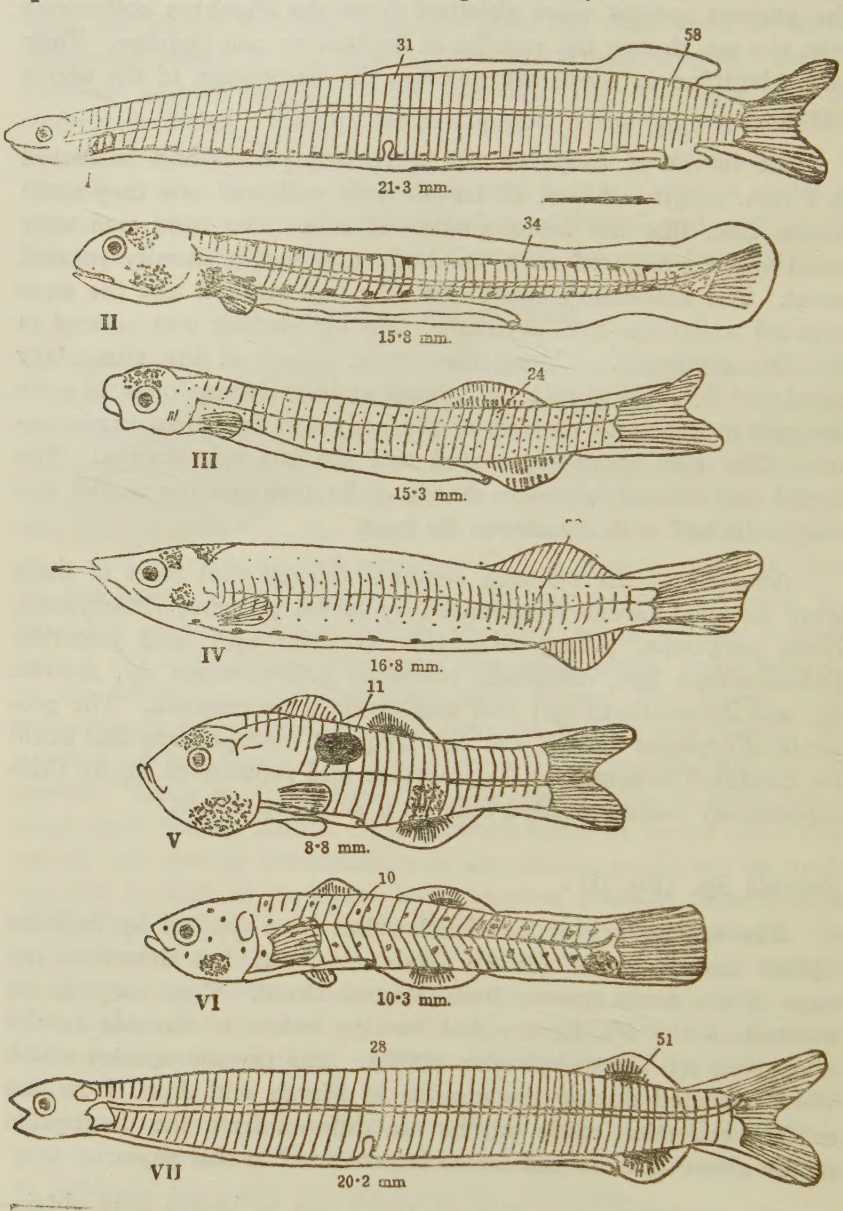
Analysis of the stomach contents showed that most of them were decapod larvae represented by larval penaeus, Brachyuran, Zoea, megalopa, young sergestids (Lucifer sp.) and pagurids. Entomostraca like, ostracods, copepods (*Paracalanus* sp., *Acartia* sp., and *Aerocalanus* sp.) and amphipods were common. The presence of copepod nauplii was also frequent. Amphipods that could be identified belonged to Gammaridae and remains of Mysis (Mesopodopsis) were recorded only in a single specimen.

Saurida Sp. (Fig II) :

The life history of lizard fishes has been studied by Raffaele (1888) and Delsman (1938). Nair (loc. cit) has described the eggs of the same species from Madras Coast. Very recently an account of the life history and feeding habits of *Saurida tumbil* was given by Vijayaraghavan (1953). The present species which were collected during the months of March and April from the surface collection made on the sea differed from the description of the above authors and hence is presented in this paper.

32 post larvae measuring 11·5-15·2 mm. in length were obtained. Excepting for the size in length, all of them were similar in appearance and other structures. The snout was somewhat pointed and the lower jaw was larger than the upper. Both the

jaws possessed well developed teeth. In front of the eye, a very prominent nostril was noticed. 34 preanal myotomes could be seen



FIGS. I to VII

through the transparency of the larvae. Fin rays were noticed in the caudal region and in the pectoral fin. Dark black patches of

pigment cells were recorded in the head region of the larvae and on the dorsal and ventral sides thick black pigment cells arranged in regular order were noticed.

Examination of the stomach contents showed copepod eggs, nauplii and adult copepods, cirripede larvae, young polychaetes, larval bivalves, species of Diatomacea, echinoplutous larvae, ceratium sp. were found in the stomachs of which copepods formed the main item of food. A single larvae measuring 11·5 mm. long was found to have fed exclusively on copepods and copepod nauplii. The common copepods identified were *Pontellopsis* sp., *Labidocera* sp., *Eucalanus* sp., *Oithona rigida, Oithona robusta, Paracalanus* sp., *Pseudodiaptomus* sp., *corycaeus* sp., *Acartia* sp., *Temora* sp., *Calanopia* sp., *Euterpina* sp. and *Macrosetella* sp. Two of the larvae were found with empty stomachs.

Stolephorus sp. :—(Fig. III)

The eggs and the larval stages of *Stolephorus* sp. occurring in the Java seas have been described by Delsman (1931). Larval and Post larval stages of *Stolephorus commersonii* have been recorded from the Madras Coast by Panikar and Iyer (loc. cit). Gopinath (loc. cit.) reported the larvae of *S. commersonii* from Travandrum Coast. The complete life history and feeding of *Stolephorus tri* from Madras Coast has been reported by Vijayaraghavan (loc. cit.). Since the present species differed from the species described by the above authors an account of the larval characters and feeding habits are given below.

26 advanced post larvae ranging from 18·5 mm. to 24·5 mm. were collected from the plankton samples from the sea during the months of February and March. Excepting the change in size there was no difference. The myotomes however were visible only on staining and clearing in Cedar wood oil. All the larvae had 24 preanal myotomes and the anus opened under the 24th myotome. The median fins were no more continuous and had developed rays, while the pectoral fins were still semi-circular and transparent. The caudal was forked. A large number of small minute black pigment spots were seen scattered all over the body of the larvae. A dark patch of pigment cells was observed in the head region about the eyes which were silvery as in the adult.

The diet of this *Stolephorus* sp. was markedly different from that of *Stolephorus tri* Vijayaraghavan (loc. cit.). It included

young and larval fishes. The nature and size of the organisms that constituted the food of this fish suggest that it was an active feeder which chased and captured its food. The stomach contents on examination was found to consist of larval forms of *Leiognathus* sp. and *Elops* sp. Most of the diet could not be made out completely due to the partial state of digestion. Partly digested bits of Polychaetes, Sagitta, *Penaeus, Acetes,* remains of copepods formed the minor items of food. Small amounts of green matter mixed with other food items were often recognized. Probably they were the digested algae or sea weeds. Previous workers like Lebour (1918) and Huxley (1888) reported that it was digested plant matter.

Hemiramphus sp. :—(Fig. IV)

Though the various aspects of Syngnathids have been widely studied outside India, the knowledge of the eggs and larvae of *Hemiramphus* and its allied forms is limited. This young gar fish which are commonly found in Madras in the months of June and July entered brackish water and fresh water occasionally. Bhattacharya (loc. cit.) and Job and Jones (loc. cit.) have described several stages of this species leading to the establishment of the adult character. Nair (loc. cit.) reported that "Post Larvae of *Hemiramphus gaimardi* had a tendency to move amidst the algae (Chaetomorpha). Very recently Vijayaraghavan (loc. cit.) has given the life history and feeding habits of *Hemiraphus* far from Madras Coast. The necessity for describing the present form arose since it differed from the post larvae described by the above authors.

The post larvae which were collected from the inshore plankton —measured 20·8 mm. in length. All the 10 larvae measured equally. The characteristic beak of the larvae was noticed. All the fins were well-developed with distinct fin rays except the pelvic fin which was indicated only by a bud like projection. The caudal fin was slightly asymmetrical owing to the dorsal lobe becoming prominent. The upper jaw formed by the premaxillaries was short and more or less triangular in form while the lower jaw was elonagated far beyond the upper. Minute veliform teeth were present in both the jaws. There were 33 preanal myotomes and the anus opened below the 33rd myotome. The post larvae were were pigmented in a very characteristic manner by greenish blue chromatophores. Three patches of greenish blue chromatophores

were observed in the head region. Small minute green pigment spots were seen scattered all over the body. Isolated more conspicuous chromatophores were noticed on the dorsal margin of the alimentary canal.

It will be seen from the analysis of the stomach contents that 50% of the crustacean consumed were decapods and nearly one third was constituted by Entomostracans, cumacea, Amphipods and Mysis. The common decapods were sergestids like *Lucifer* larval *Penaeus indicus* and larval forms of brachyuran crab like the Zoea and megalopa crustacean eggs and obstracods also appeared in small quantities. Apart from these diatoms (*Asterionella* sp., *Chaetocera* sp., *Thallasiothrix* sp. and *Cosinodiscus* sp. and Copepods were represented in very small quantities.

Ambassis sp. :—(Fig. V).

Delsman (1926) has reported the larval forms of *Ambassis* sp. while Gopinath (loc. cit.) recorded the larvae of *Ambassis ambassis* from Travandrum Coast. All these forms differed from each other. Nair (loc. cit.) from Madras waters has identified his species as *Ambassis miops* and reported that the "breeding season ranged from October to December when adult fishes with ripe gonads have been collected in large numbers both from the sea and from the Coovum and Adyar." The present species were collected from the brackish waters of Coovum and Adyar and were identified as *Ambassis* sp. in accordance with the description of the above authors. Since this glass fish differed from *Ambassis ambassis* and *Ambassis miops* an account of the larval characters and their food is given below :—

38 post larvae were collected, the smallest larvae measuring 9·2 mm. There was no difference between the larvae of 9·2 mm. and the larvae of 18·2 mm. except in length. The larvae were extremely transparent with light golden colour, the sides of the abdomen and opercles were bright silvery. The air bladder was visible through the transparant abdomen as a small bubble of air at its dorsal side. The anus was situated below the 11th myotome. Large black and highly branched chromatophores without any regularity were noticed in front of the pectoral fin and in the head region. A similar pigmentation was noticed in the region of the 15th and 17th myotome. A prominent black patch also was noticed. Fin rays began to develop in the pectoral, dorsal, anal and caudal

region. In the dorsal fin the 1st finray was very short while the second was longer and stronger than the rest.

Analysis of the stomach contents showed that the larvae fed mainly on teleostean food, such as fish eggs and pro-larvae. The eggs could not be identified due to the advanced state of digestion, whereas the larvae were identified as *Caranx* sp., *Stolephous* sp., *Engraulis* sp., *Leiognathus* sp. and *Trichiurus* sp. Apart from this, crustacea such as copepods, ostracods and Amphipods, were also recorded in the stomach in small quantities. Nair (loc. cit.) reported that for *Ambassis miops* copepods formed the main item of food, and he has also recorded that under the laboratory condition the post larvae have been observed to feed very rapidly without discrimination on micro-organisms of the plankton. Therefore it may be concluded that the data presented by Nair (loc. cit.) and the present author may be due to the difference in species as well as the difference in localities from which these fishes were caught. It is also suggested that a large number of fishes should be examined and the precentage of the different items of food calculated with a view to have an accurate generalization regarding crustacean or teleostean being the favourite item of food.

Gobius sp. :—(Fig. VI)

Delsman (1926) has given an account of the early larval stages of *Gobius* sp. from Java seas. While Gopinath (loc. cit.) has mentioned a few advanced post larvae collected from Trivandrum Coast, nothing is known about the larvae of *Gobius* except these. Both of them were not able to identify the species and both the forms differed from each other. With the help of Delsman's description (1826 a) the present forms were identified as *Gobius* sp. which differed from those of the two authors and hence is reported here, in this account.

29 specimens were collected in the months of November and December from the brackish water plankton from Adyar and Coovum. The larvae were extremely transparent measured 13·8 mm. in length and had 10 preanal and 18 post anal myotomes. The alimentary canal ran as a straight tube and opened under the 10th myotome. All the fins were well developed and fin rays began to develop. The eyes were pigmented and the mouth opening was very clear which led, to the buccal cavity. Gill openings were of moderate size. Body was low and elongated, canine teeth in both the jaws were noticed. Green pigment patches were seen

all over the body. A large patch of green cells were observed in front (below the eye) and behind (in the caudal region). Black pigment cells were seen scattered here and there in the body of the larvae.

The analysis of the stomach contents of these fishes revealed that decapod crustacea, such as anomuran larvae, megalopa and zoea stages of Brachyuran crabs formed the major items of food. *Perichimenes indicus* was the most common decapod identified. Copepods were identified as *Schmackeria annandalai, S. tollingarai, Mesocyclops hyalinus, Mesocyclops leukarti, Microcyclops biclor,* and *M. varicans.* Other crustacean were represented as *Balanus amphitrite, Mesopodopsis orientalis, Grandidierella gilese, Penaeus indicus.* The following teleostean food items were recorded in the stomach, larval form of *Stolephorus* sp., *Ambassis* sp. and *Elops* sp.

Megalops cyprinoides: (Brouss) (Fig. 7)

As soon as the bar was opened on night of November 23rd 1956 plankton collection made on the next morning in Coovum revealed the larvae of *Megalops.* Raj (loc cit.) has observed the larvae of *Megalops cyprinoides* in river Coovam in Madras during the months of October and November. The larvae of *Megalops cyprinoides* were first described by Van Hampen (1908) and later on by Delsman (1926). Gopinath (loc. cit.) has recorded the larvae of *Megalops cyprinoides* from Trivandrum Coast. The larvae were easily identified as *Megalops cyprinoides* from the descriptions of the above authors and together with the feeding habits are presented here.

All the larvae measured 24·6 mm. in length and about 24 larva were collected. The head was broader than that of the *Elops* sp. and the larvae were transparent and ribbon-shaped. The snout was not pointed. Eyes were covered with narrow adipose lids and they were pigmented. The mouth was anterior in position and the lower jaw was prominent. Villiform teeth were present in the jaws. All the fins were well formed. The dorsal fin commenced opposite the ventral. The air bladder was noticed in between the 28th and 24th myotome. There were 51 preanal myotome and the anus opened below the 51 myotome.

The analysis of the stomach contents showed that crustacen item dominated the food of these fishes of which copepods domi-

nated. The crustacea were identified as copepods, *Penaeus* sp., *Squilla* sp. and *Acetes* sp. The copepod were identified as *Mesocyclops* sp., *Acartia bifilosa, Microcyclops* sp. *Teleostean* food item was represented in a very small quantity. They were identified as pro larvae and post larvae of *Ambassis* sp., *Therapan sp.* and *Etroplus* sp.

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REFERENCES

Bapat, S. V. and Bal, D. V. (1952) The food of some young fishes from Bombay. *Proc. Indian Acad. Sci.*, 35 (2) B: 78-92.

Bhattacharya, D. R. (1916) Stages in the life history of *Gobius, Pteroserites* and *Hemiramphus* Fauna of Chilka Lake. *Mem. Indian Mus.*, 5: 383-392.

Chacko, P. J. (1949b) On the nutrition of the young stages of certain estuarine fishes of Madras. *Proc.* 36th *Indian Sci. Congr.*, Allahabad, 1949, Abstract, Calcutta, 153-164.

Chidambaram, K. (1941) Observations on the development of *Arius jella* (Day). *Proc. Indian Acad. Sci.*, 14: 502-508.

Clark, R. S. (1920) Pelagic young and early bottom stages of teleosteans. *J. Mar. biol. Ass.*, 12: 159-210.

Delsman, H. C. (1925) Fish eggs and larvae from Java seas. No. 4 *Dussumieria hasselti. Treubia*, 6: 297-307.

—— (1926) Fish eggs and larvae from Java seas No. 10. On a few larvae of Empang fishes. *Ibid.*, 8: 400-402.

—— (1922) Fish eggs and larvae from Java sea, No. 2 *chirocentrus dorab* (Forsk). *Ibid.*, 3 (1): 97-108.

—— (1929) The study of Pelagic fish eggs. *Fourth Pacif. Sci. Congr.*, Batavia (Java), 1-7.

—— (1931) Fish eggs and larvae from the Java seas No. 18. Genus *Cybium. Ibid. Pacif.* 13: 401-410.

—— (1933) Fish eggs and larvae from the Java seas 21. Eel eggs, 22. *Clupeoids lile* 23. A few more Clupea eggs. *Ibid.*, 14 (2): 237-257.

—— (1938) Fish eggs and larvae from the Java sea 24. *Mycotophoidia. Ibid.*, 16 (3): 415-420.

Gobinath, K. (1946) Notes on the larval and post larval stages of fishes found along the Trivandrum Coast. *Proc. nat. Inst. Sci. India,* **12**: 1-21.

Job, T. J. and Jones, S. (1938) Studies on the development of Indian Gar fish *Tylosurus strongylurus* (Van Hass) with notes on the young stages of *Hemiramphus gaimardi* (C & V) *Rec. Indian Mus.,* **40**: 245-253.

John, M. A. (1951) Pelagic fish eggs and larvae of the Madras coast. *J. zool. Soc. India,* **3** (1): 38-66.

Jones, S. and Menon, M. A. S. (1951) On the bionomics and development of some Indian flat fishes. *Ibid.,* **3** (1): 67-79.

Jones, S. (1937) Observations on the breeding and developmental stages of certain brackish water fishes of Adyar, Madras. *Proc. Indian Acad. Sci.,* **B, 5**: 261-289.

Kuthalingam, M.D.K. (1955a) Food and feeding habits of Juveniles and adults four fishes of Madras. *J. Madras Univ.* B., **25**, No. 3: 235-253.

—— (1955b) The food of Horse-Mackerel *Caranx. Curr. Sci.,* 24: 416-417.

Lebour, M. V. (1918) Food of post larval fishes. *J. Mar. biol. Ass.* **11**: 433-469.

—— (1919) Food of post larval fishes. *Ibid.,* **12**.

—— (1920) Food of young fishes. *Ibid.,* **12**: 261-324.

—— (1921) Larval and post larval stages of Pilchard, sprat and Herring from plymouth. *Ibid.,* **12**.

—— (1922) Feeding habits of young fishes. *Ibid.,* **12**: 9-21.

—— (1923) Food of Plankton organisms. *Ibid.,* **13**: 70-92.

—— (1924) Food of young Herrin. *Ibid.,* **13**.

—— (1925) Young Anglers in captivity and some of their enemies. *Ibid.,* **13**: 721-34.

Nair, R. V. (1952) Studies on some fish eggs and larvae of the Madras Plankton. *Proc. Indian Acad. Sci.,* **B, 35**: 181-208.

Panikar, N. K. and Iyer Gopala, R. (1937) Brackish water fauna of Madras. *Ibid.,* **6**: 284-337.

Raffaele, F. (1888) Le uova galleggianti ele larva dei Teleosti nel Golfo di Napoli. *Mitt. zool. Sta. Neapel.,* **8**: 1-84.

Rao, H. S. and Hora, S. L. (1938) On the ecology, bionomics and systematic position of the Blennid fish of the genus Andamia. *Rec. Indian Mus.,* **40**: 377-401.

Raj, B. Sundara (1916) Fresh water fish of Madras. *Ibid.,* **12**: 249-294.

Southwell, T. and Prashad, B. (1919) Embryological and developmental studies of Indian fishes. Notes from the Bengal fisheries Lab. No. 6. *Ibid.,* **16**: 215-240.

Van kampan (1908) *Larvae of Megalops cyprinoides.* Bull. Dép. Agric. Ind. néerl. **20**.

Vijayaraghavan, P. (1953) Life history and feeding habits of some Madras fish. (unpublished thesis).

Life history and feeding habits of the spotted seer *Sconsbermorus Guttatus* Block and Schneider. *Indian J. Fish.,* **2**: 360-372.

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The Food and Feeding Habits of Some Young Silver-Bellies

BY

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ABSTRACT

1. The stomach contents of 370 juveniles or young fishes belonging to four different species of Silver-bellies are reported on.

2. All the four species, *Gazza minuta, Leiognathus insidiator, Leiognathus daura, and Leiognathus splendens* are surface feeders during their early life. The diet of these young fishes when compared with those of the adults show that in *Leiognathus splendens* there is no change of diet except what is to be expected from the change in size; that *Gazza minuta* changes from a plankton-feeding habit to a bottom-feeding habit; that the young of *Leiognathus daura* filter surface-living protozoa and that *Leiognathus insidiator* feeds on all the forms in the plankton and when it becomes older confines itself to only teleostei.

Introduction

Very little is known regarding the food and feeding habits of young fishes of Indian Coast. The food of early stages of 37 species of fishes of Bombay has been reported on by Bapat and Bal (1950 and 1952). Gopinath (1942) has given a brief account of the feeding habits of four species of post larval fishes of Trivandrum Coast.

The Silver-bellies constitute one of the important food fishes of the Madras Coast. Moses (1923) would even rank them formost in the economic grade. Therefore the food and feeding habits of young Silver-bellies belonging to the species *Leiognathus daura, L. splendens, L. insidiator* and *Gazza minuta* are treated in the present paper.

Material and Method

The material consists of 18 post larvae and 352 juveniles collected from the plankton and from the fishermen's nets both in

the morning and evening. As soon as the fishes were collected they were identified, measured and their stomach contents were analysed. Since the young ones were too small for dissection, the gut contents were gently pressed out through the anus into a watch glass. The gut contents were washed with a measured quantity of water. After a qualitative analysis of the gut contents a quantitative estimate of the stomach contents was made. A glass tube with an internal diameter of 2·5 mm. and about 15 mm. long was fitted with a rubber teat and was graduated into divisions containing ·01 cc. The gut contents of each juvenile fish were measured volumetrically to the nearest second decimal place with the help of such a graduated tube.

Leiognathus insidiator: (Bl)

According to Smith (1951) the distribution of *Secutor insidiator* is very wide extending over central tropical and Indo Pacific regions. In the inshore area of Madras the Juveniles occur from July to April. They are dusky coloured above, silvery below, the back with irregular vertical series of darkish spots, fins yellow like those of the adults; the top of the dorsal fin is marked by a black patch.

A total number of 103 juveniles ranging between 1 cm. to 3·5 cm. were collected. Out of this, except seven, all had food in the stomach.

The stomach contents were analysed, and the undigested and unidentifiable constituents were separated and expressed in percentages of the total volume of food and the data obtained are given in the table on p. 15.

From this table it will be seen that crustacea form the main food item of the fish and that copepods form the bulk of the crustacea. The copepods were identified to be (1) *Eucalanus sp,* (2) *Eucalarus elongatus,* (3) *Eucalanus attenuatus,* (4) *Rhincalanus cornutus,* (5) *Paracalanus parvus,* (6) *Paracalanus aculeatus,* (7) *Acrocalanus longicornis,* (8) *Acartia sp,* (9) *Oithona sp.,* (10) *Oithona rigida,* (11) *Oithona spinulosa,* (12) and *Corycaeus sp.*

Sagitta were found in the fish in all the months except January. Larval bivalves and gastropods were found always though, Pteropods occurred only occasionally. In July, September, December and March crustacean eggs were met with. The crustacean eggs were mostly those of copepoda. Very small quanti-

Table showing the average total volume of food and also the percentage average of the various food items of Leiognathus insidiator

Particulars.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.
Volume of stomach contents in c.c. ..	0·05	0·03	0·04	0·04	0·03	0·04	0·02	0·03	0·04	0·04
Copepods ..	27·8	55·0	35·7	66·4	43·3	31·4	65·1	41·3	47·0	75·3
Other Crustacean remains ..	51·1	34·4	38·7	3·7	10·7	31·4	27·0	25·0	18·2	14·9
Sagitta ..	10·0	4·5	13·7	12·9	21·8	11·3	—	12·0	12·2	6·6
Molluscans ..	10·0	6·1	10·5	9·5	21·8	15·9	2·6	17·0	15·7	3·2
Eggs ..	1·1	—	0·7	—	—	6·3	—	0·7	4·6	—
Other remains (Algae, Zoa, Diatoms) ..	—	—	0·7	7·8	2·4	3·7	5·3	4·0	2·3	—

ties of zoea, algae and diatoms were present in the stomach. It is possible that the small quantities of sand particles present, were accidentally taken in along with food.

Leiognathus daura (*cuv*) :—This fish is known as "Namakarel" in Tamil. Distributed in seas of coromandel coast, Ceylon and Malaya Archepelago (Day, 1889). Out of 73 fishes examined except 8 all had food in the stomach. The fishes examined ranged from about 1 cm. to 3 cm. in length. They occurred in maximum numbers in the month of January.

Taking only the undigested and identifiable matter of the stomach contents into consideration the volume of food and also the percentage averages of the stomach contents are presented in table on p. 17.

From the data tabulated above it can be seen that protozoa form the main bulk of the food. Radiolarians, Noctiluca, Ceratium sp, Dinoflagelatta, Tintinnids were identified. Copepods were represented by *Oithona sp*, and *Acartia sp*. Other crustacea were represented by larval lucifer and Decopods. Larval gastropods, bivalves and pteropods constituted the Molluscan items of food. Occasionally diatoms belonging to the genus *Chaetoceros*, *Bacteriastrum* and *Thallasiothrix* were found. Of these, *Chaetoceros*, is the commonest. In the months of August and November the stomachs contained a few teleostean eggs. Partly digested glass arrows were also met with occasionally. The small quantities of sand found occasionally in the stomachs must have been inside the gastropods fed upon.

Gazza minuta (*Bloch*) :—This Carangid which occurs in the inshore waters of the Madras coast throughout the year, is one of the commonest fishes sold in the Madras market and is known as "Kotookaanal". 101 juveniles were obtained from the fishermen's nets from August to April. They ranged from 1 to 3 cm. in length. Of these 94 had food in the stomach. The volume of food and also the percentage average of the stomach contents are shown in table on p. 18.

From the table it will be obvious that while crustacea, mollusca, *Sagitta* and fish eggs formed nearly 84% of the food, vegetable matter makes up the rest of the diet. Therefore it is safe to infer that the juveniles of *Gazza minuta* are omnivorous. The vegetable matter was mostly digested but from the remnents one can see that diatoms and algae are fed upon. Of the animal matter

Table showing the average volume of food and also the percentage average of the various food items of Leiognathus daura

Particulars.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Vol. of stomach contents in c.c. ..	0·06	0·04	0·05	0·04	0·04	0·03	0·03
Protozoan items ..	66·4	37·4	41·6	46·3	56·2	61·0	67·7
Copepods ..	6·1	25·8	24·5	15·7	14·8	13·7	9·5
Crustacean remains ..	5·6	9·7	20·0	16·7	19·5	11·0	15·2
Molluscan items ..	9·0	7·5	3·2	10·9	7·0	3·3	4·2
Vegetable matter ..	9·0	6·4	2·7	8·0	2·5	4·0	3·4
Fish eggs ..	0·6	—	—	0·6	—	—	—
Miscellaneous items (Lucifer, Decapods and sand particles) ..	3·3	3·2	8·0	1·8	—	7·0	—

Table showing the average volume of food and also the percentage average of the various food items of Gazza minuta

Particulars.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apl.
Vol. of stomach contents in c.c. ..	0·04	0·07	0·06	0·04	0·03	0·05	0·06	0·05	0·05
Copepods ..	35·1	32·0	55·4	37·8	46·3	41·8	14·7	42·6	72·1
Crustacean appendages and other crustacean remains ..	43·8	9·6	2·6	27·0	10·3	28·2	32·5	29·6	13·0
Vegetable matter (Diatoms, Algae and green matter) ..	7·5	37·8	11·7	13·6	10·3	11·5	44·8	8·4	4·8
Zoea ..	2·7	6·5	13·7	5·4	7·8	5·0	3·3	—	2·0
Molluscans ..	7·0	5·8	2·3	5·4	7·8	3·6	3·5	12·1	2·3
Sagitta ..	2·9	5·8	8·2	5·4	11·3	6·6	—	1·6	4·6
Fish eggs ..	1·8	2·5	1·3	5·4	6·2	3·3	1·2	0·7	1·2
Other remains (Pteropods, Decopods and Lucifer) ..	1·2	—	4·7	—	—	—	—	5·0	—

crustacea formed the main bulk. Copepods were represented by (1) *Eucalanus* sp, (2) *Paracalanus sp,* (3) *Schmackeria sp,* (4) *Acartia erythraea,* (5) *Euterpina acutifrons* (6) *Oithona rigida* (7) *Oithona robusta,* (8) *Oithona spinulosa* and (9) *Corycaeus* sp. *Zoea* and *Mysis* also occurred in appreciable numbers and there were remains of Decapods and *Lucifer. Sagitta enflata* occurred in the stomach contents during most months forming nearly 5·8% of the volume. Mollusca were represented by larval bivalves and gastropods and occasionally by Pteropods. Fish eggs were always present though owing to the nature of the size they formed but a small percentage of the total volume. The inclusion of gastropods in the diet is probably responsible for the occasional presence of trace of sand particles.

Leiognathus splendens (Cuv).

In Madras the juveniles of this species are caught from September to December and are marketed. They are known as "Kalikaral".

93 juveniles ranging between 1 to 3·5 cm were collected. Of these 8 were without food. The data obtained through analysis are presented in the following table:

Table showing the average volume of food and also the percentage average of the various food items of Leiognathus splendens.

Particulars.	Sept.	Oct.	Nov.	Dec.
Volume of stomach contents in c.c. ..	0·04	0·03	0·05	0·04
Copepods ..	40·2	29 8	30·8	58·2
Prawn larvae ..	8·3	3·4	—	1·4
Amphipods ..	8·2	—	2·2	2·4
Algae ..	8·6	—	3·4	—
Polychaetes ..	0·8	—	—	2·3
Molluscans ..	10·4	10·2	4·8	—
Zoea ..	1·1	2·7	—	—
Crab remains ..	2·3	—	—	0·8
Crustacean remains ..	21·1	53·9	58·8	34·9

It is evident from the above table that copepods such as (1) *Eucalanus sp,* (2) *Paracalarnus sp,* (3) *Paracalanus parvas,* (4) *Paracalanus aculeatus,* (5) *Pseudodiaptoms sp,* (6) *Euterpina,* (7) *Acartia sp,* (8) *Aerocalanus longicornis,* (9) *Oithona sp,* (10) *Oithona rigida* and (11) *Corycaeus sp,* form a considerable proportion of the diet together with Prawn larvae and Amphipods. Though copepods seem to be the favourite food of this species during the early stages of development there appears to be no specific preference to species or genera of copepods. The diet included a small trace of Polychaetes which were, however, unidentifiable. The molluscs forming nearly 9 % of the diet were chiefly of bivalves and gastropods and occasionally pteropods. Filaments of Algae and green matter formed a relatively small fraction of the total volume of food.

Discussion

The analysis of the stomach contents of the young ones of *Gazza minuta* would show that they are surface feeders. They are omnivorous and feed on planktonic crustacea, diatoms, algae, larval molluscs, gastropods and *Sagitta.* The adults, according to Vijayaraghavan (1950, unpublished thesis) feed on a large amount of foraminifera and sand particles from the bottom of the sea, as well as on *Penaeus indicus, Acetes erythraeus,* crab, copepods, zoea, megalopa and other small forms found in the surface waters. He concludes from these facts that the adults live in shallow regions of the sea where they can move freely up and down. It will be obvious from the data collected and presented here by the author that the juveniles are only surface living forms and do not visit the sea bottom. It may therefore be concluded that when the juveniles become the adults they frequent the sea bottom as well.

The juveniles of *Leiognathus insidiator* are surface-living forms and feed on the zooplankton such as copepods, *Sagitta, zoea,* megalopa stages of Brachyuran larvae, larval bivalves and gastropods. Vijayaraghavan (loc. cit), who examined only 11 adults suggests that in the adult stage this fish takes chiefly crustacean food. Whereas an examination of 33 adults of the same species by the author showed that teleostei form 88·5 % and crustaceans form only 11·5 % of the food. Since the adult fishes stay in Madras inshore waters only in the month of August, it is probable that they feed on either crusacea or fish larvae, whichever may be found, in the area. The adult fishes examined by the author

showed that these fishes live on larvae and juveniles of *Stolephorous tri,, Engraulis gravi, Polynemus sextarius* and *Mugil troschelii.*

The juveniles of *Leiognathus daura* also are plankton feeders. But these in addition to the crustacean items such as copepod, zoea and Megalopa stages of Brachyuran larvae, feed chiefly on planktonic protozoans such as tintinids, dinoflagellates, Radiolaria, *Noctiluca* and *Ceratium* sp., Since Vijayaraghavan (loc. cit.) had examined nearly 75 adult specimens the author did not study the adults. Vijayaraghavan's data on the food of adults and *Leiognathus daura* show a definite partiality for crustacea such as copepods, ostracods, *Penaeus indicus, Acetes erythraeus, Squilla sp,* and *Lucifer.*

Considering the marked preponderence of the pelagic or surface-living protozoa in the food of young fishes it is probable that the juveniles have a partiality for this food item. It may also be concluded from the foregoing, that with the growth in size, *Leiognathus daura* changes its diet and shows a definite preference to crustacea.

The juveniles of *Leiognathus splendens* subsist mainly on a diet of copepods as well as other small larval crustacea found in the surface waters. Vijayaraghavan (loc. cit.) had noticed that the adults prefer larger crustacea such as *Penaeus sp, Acetes erythraeus* and *Lucifer* as well as smaller crustacea like copepods and other larval forms. From this it can be concluded that *Leiognathus splendens* perhaps does not exhibit any marked difference in its feeding habits during its growth.

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REFERENCES

Bapat, S. V. and Bal, D. V. (1950) The food of young clupeiods. *Proc. Indian Acad. Sci.*, **B**, **32**, 39-58.

—— & —— (1952) The food of some young fishes from Bombay *Ibid.*, **35** (2) B: 78-92.

Gopinath, K. (1942) Distribution and feeding of the Post-larval fishes of the Trivandrum coast. *Curr. Sci.*, **11**: 337.

Kuthalingam, M. D. K. The food of Horse Mackerel *Caranx djedaba*, *Curr. Sci.*, (in press).

Moses, S. T. (1923) A statistical account of the fish supply of Madras. *Madras Fish. Bull.* 131-166.

Smith, L. R. J. (1951) Thirteen noteworthy additions to South East Africa Marine fauna. *Ann. Mag. not. Hist.*, 12th Ser. 4 (37): 49-66.

Vijayaraghavan, P. (1950) Food of a few common fishes of the Madras coast. M.Sc. Thesis submitted to the University of Madras.

—— (1953) Studies on fish eggs and larvae of Madras coast. Ph. D. Thesis submitted to the University of Madras.

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The Development and Metamorphosis of Three Species of Sessile Barnacles

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ABSTRACT

The early development, duration of larval life, and the complete descriptions of six naupliar and cyprid stages of *Balanus amphitrite variegatus* are given.

The attachment of the cyprid larva and the different phases of orientation are dealt with in detail.

The developmental stages of *Balanus tintinnabulum tintinnabulum* are briefly sketched and are compared with that of *B.a. variegatus.*

The developmental stages of *Chthamalus stellatus stellatus* are described and the salient differences like size, duration of larval life for the same species described by Bassindale (1936) are given.

Introduction

The nauplius and cypris stages in the development of barnacles were first noted by Thompson (1830-35) and Burmeister (1843) but the different larval stages of the Pedunculate barnacle (*Lepas fascicularis*) were described by Willemoes-Suhm (1876) and those of the operculate barnacle *Balanus perforatus* by Groom (1894 a). The transformation of the cypris into adult was briefly described by Darwin (1854), Pagenstecher (1863) and Claus (1869) while the details of the formation of the valves of adult were supplied by Herz (1933), Nilsson-Cantell (1921), Runnstorm (1925, 26), Kunhert (1935), Bassindale (1936), Lochhead (1936), Yasugi and Ishida (1937), Batham (1946), Pyefinch (1948 a & b, 1949) Knight-Jones and Waugh (1949), Norris and Crisp (1953-54), Sandison (1953), and Jones and Crisp (1953-54) further added to our knowledge of the free swimming stages in the development of the cirripedes of the temperate waters, though no author has investigated and described completely the development and metamorphosis of the cypris.

On the other hand, practically nothing is known about the development of barnacles inhabiting the tropical regions. There-

fore it was considered worthwhile to study the development of some of the common barnacles, (*Balanus amphitrite variegatus, B. tintinnabulum tintinnabulum* and *Chthamalus stellatus stellatus*) in the course of an investigation of the biology of fouling organisms of Madras.

Mature barnacles were kept alive in large troughs in the Laboratory. Daily renewal of seawater ensured a healthy condition of the animal for several weeks and facilitated the observation of the copulation and subsequent changes, in samples of the same age. Individuals isolated and kept in separate containers and examined periodically proved incapable of self fertilization, even for as long periods as six months. This was the case in all the three barnacles studied. But when they were kept in large numbers in the same container, copulation was observed and the individuals which had just copulated were dissected and the eggs were taken into petridishes of fresh filtered seawater. From observations made in the laboratory it was possible to time the hatching of the nauplius and the different moults the nauplius undergoes. Collections from the field also were used for confirming the duration of the different periods of development under laboratory conditions. They were invariably found to be identical.

In all the three barnacles studied, it was found that there were six distinct naupliar stages and one cypris stage as in the barnacles inhabiting the temperate regions, though the duration from fertilization of the ova to the liberation of the 1st stage nauplius and that of the different stages has been greatly reduced so that the total time taken for development is much abbreviated. The setation formula elaborated by Bassindale (1936) with a view to facilitate a comparison of the nauplii of the different species of barnacles, is not found to be of much practical use in this study, since the pattern of naupliar setation appeared to be similar although the adults were distinct. In the present study, the details of the changes in structure and orientation of the organs were obtained by paraffin sections.

(ii)

(a) *Balanus amphitrite variegatus.*

This subspecies reproduces throughout the year. Copulation and liberation of larvae were observed in all the twelve months. From a study of the life histories of several barnacles settled on

fresh panels left in different sites on the coast, the author found that this subspecies attains sexual maturity 14 days after the metamorphosis of the cyprids. Upto the 7th day the gonads appear whitish and contain spherical oocytes. In 8 day old barnacles these cells become elongated and are filled with yolk granules and about the 11th day the ova are led along the oviducts through "the auditorial sac" (Darwin) and deposited at the sides of prosoma and covered over by the ovigerous lamellae within the mantle cavity. A secretion from probably the ovary holds the ova together in a gelatinous mass. These events were followed in barnacles reared in the laboratory as well as those collected from the harbour.

The ripe unfertilized egg (Pl. 1. Fig. 1) is light grey in colour, ellipsoidal in form and measures 0·07—0·09 m.m. It is covered by vitelline membrane and entirely filled with yolk-granules. A barnacle is frequently seen copulating with a number of others in rapid succession. One such barnacle which had impregnated was taken and the penis squeezed on a slide. The whitish fluid thus obtained contains the sperms which are hairlike and filamentar. Into this whitish sperm-fluid ripe unfertilized eggs from another barnacle were introduced. Within 40 minutes, the colour of the egg became yellow and the cytoplasm became more granulated. Two tiny polar bodies appeared towards the periphery of the now opaque egg. But the formation of a thick envelope outside the vitelline membrane as well as the other changes observed indicated that fertilization had taken place. Two hours after the introduction of the eggs into the sperm-fluid the yellow colour of the egg gradually becomes brown and when stained with neutral red shows faint lines of cleavage. The brownish colour darkens to a russet colour and appendages could be distinguished by the second day. (Pl. 1. Fig. 2). About the third day the embryo shows signs of movement and roughly about the 96th hour the egg membranes are ruptured along the longitudinal axis and the nauplius thrusts its appendages through the rupture and forces its way out. (Pl. 1. Fig. 3). The straightening of the appendages of the nauplii kept in a curled position probably assists in the rupture of the egg membrane.

Copulation was observed in a number of individuals on several occasions in the laboratory and in all these cases the larvae emerged from the mantle cavities of the parents, 96 hours after copulation. Hence it was concluded that the mass of eggs which were fertilized *in vitro* should have undergone similar develop-

ment. The nauplii hatched from the egg in this way resembled in all respects the nauplii which were found emerging in a steady stream out of the mantle cavity of adult barnacles in the field.

These first nauplii swim about in the containers, congregating towards the side nearest to the window. The carapace is rounded anteriorly with the convex lateral sides tapering to a point posteriorly (Pl. 1. Fig. 4.) so that the outline is broadly oval. The frontal margin is produced laterally into two horns directed obliquely downwards. The carapace measures 0·11 mm. to 0·13 mm. broad at its greatest width just behind the frontolateral horns. About the middle of the front margin of the body, a pair of flexible structures, called the frontal filaments are found. At the posterior end of the carapace there are two processes, the abdominal process extending straight behind and bifurcating into two spines, and the postero-dorsal process or the caudal spine. In this early stage of the nauplius these processes are exceedingly short. The nauplius eye is square in outline and occurs medially about 5 to 10 μ behind the anterior margin of the carapace. It is surrounded by a tissue having a bilobed form. The oral lip is situated immediately posterior to the eyespot. The alimentary canal is a simple straight tube leading from the mouth to the anus which is situated in front of the caudal spine.

The first pair of appendages — the antennules are uniramous and four-segmented. The three basal segments are rectangular in shape while the distal segment is very small and conical and bears four long setae. A spine occurs on the distal margins of the first and the second segments and two short setae on the third segment.

The second pair of appendages or antennae are biramous. The protopodite is two-articled and bears a masticatory hook on its basal segment. The 5-jointed exopodite is devoid of setae on the dorsal margin. The distal segment carries a terminal long seta, while each of the other segments bear a long characteristically curved seta at its ventral corner. The endopodite is 4-jointed, with the distal-tip bearing two long setae and one short spine and the tips of the first, second and third segments bearing a pair of short setae on the ventral margin.

The third pair of appendage or mandible is also biramous. At the base of the two-segmented protopodite is a masticatory hook which is curved and directed downwards. The exopodite is

4-jointed with the distal tip bearing a slender long seta and the ventral margins of the other three segments bearing a long slender seta each. The endopodite is 4-jointed with the tip of the 4th segment bearing three long setae and the terminal region of the 1st, 2nd and 3rd segments bearing a pair of setae. The setae of all the appendages lack setules in the nauplii of this stage.

The Second nauplius (Pl. 1. Fig. 5.)

Within 40 minutes after hatching the first stage nauplius moults into the second stage nauplius. The caudal spine and abdominal process are now greatly elongated and the fronto-lateral horns which were directed obliquely downwards are now broader and longer, slightly curved and directed almost at right angles to the lateral sides (as in figure). The carapace has broadened to 0·14 to 0·15 mm. and the anterior margin has become more convex.

The antennule has not changed except in many of its setae now possessing setules. The exopodite of the antenna is 7-jointed the distal end of the 7th segment bearing one long naked seta and the distal tips of the first to the sixth segments each bearing one long serrated swimming seta on the ventral side. The endopodite has not changed except for its setae becoming serrated. The exopodite of the mandible has one more segment without any setae added at the base, and all the setae are now serrated. The first and third joints of the endopodite now carry a long slender seta as well as a short stubby seta on their ventral margins; the second segment bears a serrated spine and a pair of simple setae and the distal segment carries three long simple setae. After swimming about for 3 hours, the second stage nauplius moults into the third nauplius.

The Third nauplius (Pl. 1. Fig. 6.)

This larva appears more robust and has a carapace breadth of 0·16 mm. to 0·18 mm. at its greatest width. The fronto-lateral horns which were hithero slightly curved become almost straight and project out from the lateral sides. The carapace bears a pair of spines posteriorly where the caudal spine joins it. The antennule has now developed a seta on the dorsal side. The exopodite of the antenna bears at its distal end 4 setae while the endopodite does not show any change. The exopodite of the mandible has acquired one more joint, and bears an additional seta. The endopodite as before, continues to be 4-articled.

Of the larvae hatched directly from the eggs some took 5 hours, some 6 hours, others 7 or 8 hours, to moult from the 3rd nauplius stage on to the 4th under the same laboratory conditions, suggesting individual variability. It was found that on many occasions a few larvae did not metamorphose even after 8 hours but continued to live for even 36 hours without undergoing any change. These larvae finally perished. It would appear that several changes in the internal organisation of the larva have to be completed before moulting can occur and that they take, five hours in some larvae and a longer period in others, and that if they are not completed within 36 hours, normal physiological processes become so delayed that death ensues. This variability in the duration of this stage cannot be related to laboratory conditions offered to the larvae since the majority moult normally within five hours, but must be ascribed to individual variations.

The fourth nauplius (Pl. 1. Fig. 7)

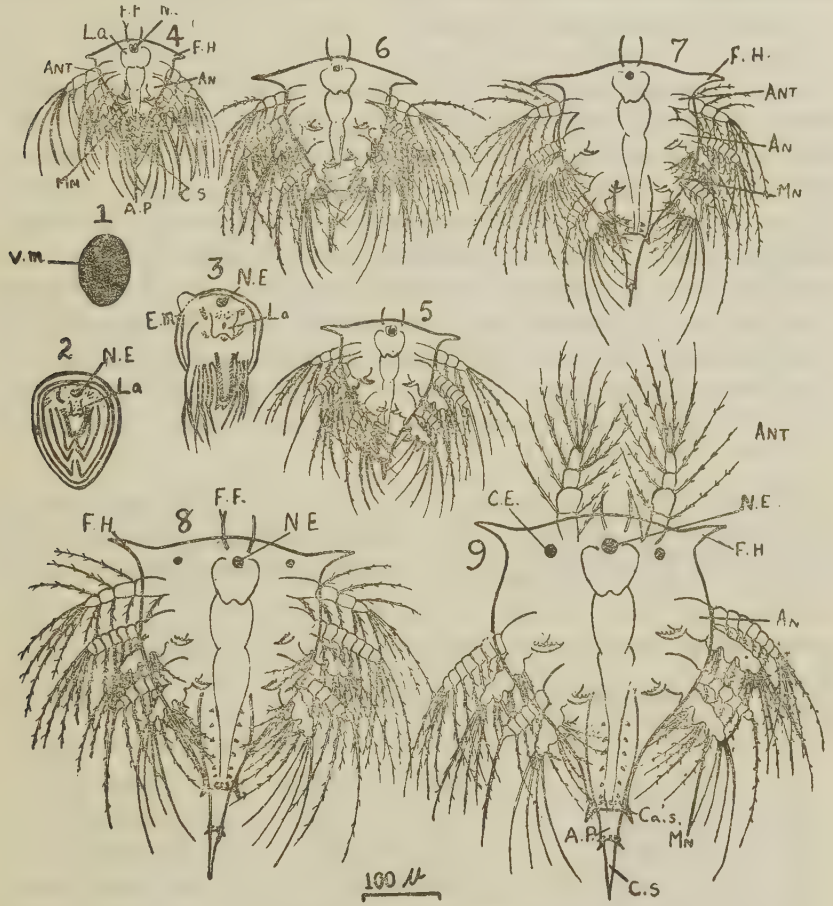
The carapace measures 0·21 mm. to 0·24 mm. between the frontal horns. It is deeply convex dorsally but fails to cover the entire body which increases in length and width projecting behind and downwards. The base of the abdominal process becomes distinctly swollen and contains the rudiments of the post mandibular appendages. On this posterior extension of the body two pairs of spines indicate the formation of the thoracic segments.

The three pairs of appendages have become conspicuous. In the antennule two more setae are developed on the dorsal side and one more on the ventral side. The exopodite of the antenna remains unchanged while the endopodite bears an additional seta terminally, and the first and third segments bear an additional seta each. The exopodite of the mandible also bears the same number of setae as in the previous stage, while in the endopodite, an additional seta is present terminally and the first and second segments bear an additional seta each. Majority of the fourth stage nauplii moult into the fifth stage nauplii at the end of four hours, but the rest do so before 8 hours are over.

The fifth nauplius: (Pl. 1. Fig. 8)

The carapace measures 0·27 to 0·29 mm. at its greatest width. This stage differs from the previous stage in that the base of the abdominal process is more swollen and now bears six pairs

of small spines on its ventral surface. At this stage in addition to the median eye a pair of lateral eyes make their appearance. Within this swollen area the rudiments of the post mandibular appendages are now more clearly discernible and each rudiment now



I. *Balanus amphitrite variegatus*

1. Unfertilized egg. 2. Fertilized egg. 3. Ist nauplius larva just emerging out. 4-9. Stages of nauplius.

An—Antenna; Ant—Antennule; Ap—Abdominal process; Ca.S.—CE—Compound eye; C.S.—Caudal spine; EM.—Egg-membrane; F.F.—Frontal filament; F.H.—Frontal horn; L.a.—Labium; MN.—Mandible; N.E.—Nauplius eye; V.m.—Vitelline membrane.

possesses a tuft of fine setae. The position of each of the six small spines on the external surface roughly corresponds with the tip of an appendage rudiment within. The frontolateral horns project forwards and outwards.

The setation formula is similar to that of the preceding stage. This nauplius swims about for an hour and moults to give rise to the sixth and last stage of nauplius.

The sixth nauplius: (Pl. 1. Fig. 9)

In this stage the body is more bulbous and has a maximum carapace breadth of 0·34 to 0·36 mm. The paired eyes in addition to the median eye are present in this stage also. The setation and the abdominal process of this stage remains the same as in the fifth nauplius stage. A bivalve shell now appears on the right and left sides of the larva and are hinged immediately below the naupliar shield. These valves, however, are so small that they do not cover the sides of the naupliar body. The six rudimentary thoracic appendages acquire swimming hairs and the compound eye becomes functional. After swimming about for eight hours the sixth stage nauplius moults into the cypris larva (i.e.) about 24 hours after hatching.

To facilitate a comparison of the change in the setation of the six nauplii, their setation formulae are given below. The setation formula has been derived by the method elaborated by Bassindale (1936).

Naupliar stage	Antennule	Antenna	Mandible
1.	0.4.2.1.1.	0.1.4-0.3.2.2.2.G	0.1.3-0.3.2.2.2.G
2.	0.4.2.1.1	0.1.6-0.3.2.2.2.G	0.1.3-0.3.2.3.2.G
3.	1.4.2.1.1	0.4.6-0.3.2.2.2.G	0.1.4-0.3.2.3.2.G
4.	1.1.1.4.2.1.1.1	0.4.6-0.4.3.2.3.G	0.1.4-0.4.2.4.3.G
5.	1.1.1.4.2.1.1.1	0.4.6-0.4.3.2.3.G	0.1.4-0.4.2.4.3.G
6.	1.1.1.4.2.1.1.1	0.4.6-0.4.3.2.3.G	0.1.4-0.4.2.4.3.G

The Cypris Larva (Pl. II. Fig. 1) has a bivalve shell, elliptical in form, 0·62 to 0·68 mm. long. The valves enclose the entire body and appendages, and an adductor muscle, in the same position as in the adult, serves to bring the valves together. The uniramous 4 articled antennule is very short and bent on itself. The basal segment is directed posteriorly while the remaining three segments of the appendage are bent forwards, forming an angle

of about 40° with the base. Two muscle bundles which take their origin from the inner surface of the wall of the basal segments run from the basal segment to the terminal segment. The body wall is retracted medio-frontally between the basal segments of the antennules of the two sides. The carapace appears connected anterolaterally to the basal segments of the antennule by a double ridged extension of chitin (described by Darwin as UU fold). This serves to fix the antennule in the acutely flexed condition, we find. The second segment is stouter, with the distal end broader than the proximal end and bears a single spine. The anterior end of the third segment is expanded into a suckerlike flat disc, used for fixation. In the centre of the disc is a six-rayed groove. The margin of the disc is fringed by six spines. The terminal or fourth segment is very minute and hook like and is attached to the side of the 3rd segment and bears three plumose setae of different lengths and a pair of recurved spinelike setae. A large olfactory filament (aesthetas) is also present.

The cement gland is sac-like whose wall consists of flattened cells and lies at the base of antennule. The long neck or duct of the gland penetrates the base of the antennule and runs through its entire length to open outside at the centre of the star shaped groove. The secretion from this gland is whitish and the composition of this fluid is of such a nature as to become adhesive in character on contact with sea water. Pyefinch (1948 b) has pointed out that the setting of this cement might take place as a result of reactions outlined by Pryor (1940).

The hinder, rounded margins of the basal segments of the antennule are inflected inwards, "forming the apodemes" the outer arms of which carry the compound eyes at about a third of the length from the anterior end of the body. Each is 50 μ in diameter and has about 7 to 10 lens units. Just towards the dorsal side of the eye and almost touching it persists the median nauplius eye now rather elongated.

The antenna which was present in all the naupliar stages is completely absent.

The mouth-parts are not only small but also much modified. The mandible is made up of two flat leaflike structures pressed close together placed side by side, one being slightly larger than the other. The first maxilla is reduced to a single leaflike structure of a more or less a rotund outline whereas the second maxilla

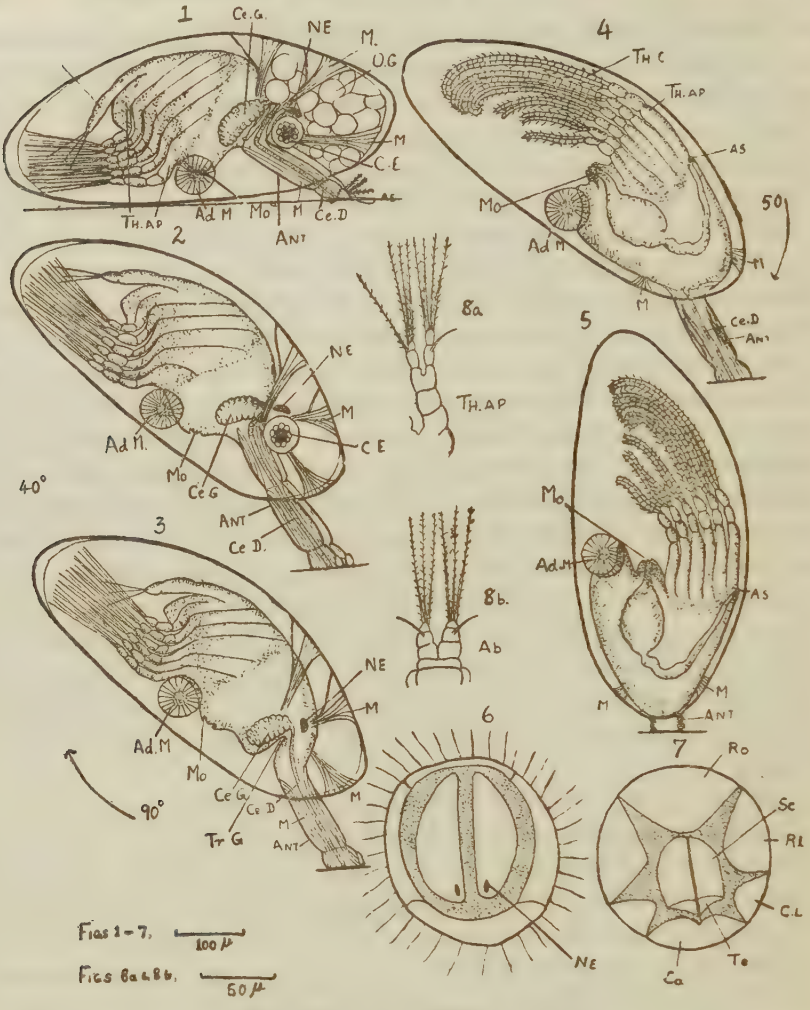
which is also leaf-like has an acuminate tip. The mouth is an elliptical opening situated in front of the maxillae bounded on either side by the mandibles. It is overhung by a short labral plate. A shrivelled tube, the oesophagus ends blind posteriorly. There are six pairs of biramous thoracic appendages directed backwards. Each is attached by a muscle bundle. In the first pair, (Pl. II. Fig. 8 A) the protopodite is of two cylindrical, unequal segments, the distal being longer. The exopodite consists of two joints of which the proximal bears at its tip a stout spine like bristle. The distal joint is leaflike and bears at its posterior margin four long setae which are longer than the rest of the leg. The endopodite also is 2-jointed with the distal segment bearing only 3 long setae, and the proximal segment hears a long plumose setae. In the second to the sixth pairs the exopodite has one more plumose setae. Since the base of each leg is attached by a stout muscle-bundle inclined forwards, the contraction of the muscle, jerks the leg backwards. Through the transparent shell, the arrangement of the sharply bent bundles and the backwardly directed legs are easily seen and serve to distinguish the larval cyprids from the Ostracods. The posterior tip of the thorax ends in a slender, short, three jointed abdomen. (Pl. II. Fig. 8 B). The proximal segment is cylindrical and long while the remaining segments are short, the terminal bearing the caudal fork. Each ramus of the caudal fork bears three long setae at its tip. Occupying nearly the anterior third of the carapace (i.e.) in front of the eye between the bases of the antennule, above the oesophagus and among the muscles of the appendages are innumerable oil globules which probably sustain the gutless cyprid.

The cyprid larva leads a free-swimming life for a maximum period of 14 days though when conditions are favourable it gets attached even after 48-72 hours. During this period it swims as well as "walks" on its antennule. The swimming movement is effected by displacing the water by means of the rapid outward and backward movements of the thoracic appendages, which result in the larva moving forwards. At times it comes into contact with the substratum (vertical or horizontal) and moves parallel to the surface using the left and right antennule to touch the surface alternatively giving the appearance of "walking" on the substratum. But the antennules are directed forwards away from the body, so the weight of the body is sustained in equilibrium and is moved forwards by the natatory movements of the cirri.

When one watches the cyprid with a lens during this period one would find that as the larva swims along, the antennal discs touch the different spots of the substratum as if "testing" them. Even when conditions are apparently satisfactory as regards the primary film, illumination, attraction of previously settled barnacles etc., the cyprid does not settle till 48 hours are over. This period is probably necessary for the differentiation and activation of the cement glands. The provision of a reserve food store in the form of fat globules though a precocious measure to sustain the larva up to a period of 14 days, if the conditions are not satisfactory, probably acts as a factor preventing settlement earlier than 48 hours. This behaviour of the cyprid namely of not settling for 48 hours, may be an adaptation to ecological factors absolutely necessary for settlement, for, as has been shown the formation of a primary film on any fresh surface takes at least 48 hours. If conditions continued to be unfavourable and the reserve store is rapidly depleted the rate of mortality among the cyprids increases after 11 days. Only about 10% of the larvae survive till the 14th day as was found by experiment. If, on the other hand all the conditions of the substratum and the environment are "satisfactory" and if 48 hours are over, the larva attaches forthwith, with its ventral surface parallel to the substratum (Pl. II. Fig. 1).

The actual process of attachment takes from 7 to 15 minutes, the cyprids varying among themselves in the duration as well as the firmness of attachment. In some, the attachment effected is firm enough even after 7 minutes while in others even after 12 to 13 minutes the attached cyprids can be dislodged by pipetting water. It is probable that the quantity of the cement secreted and the quality or setting nature of the secretion may vary in different individuals. The termination of this process of attachment is evidenced by the entire body of the larva being lifted upwards, on its antennal stalk to form an acute angle with the substratum (Pl. II. Fig. 2). This lifting of the body is always against the current if there is one. As the lifting takes place even in still water, it must be due to a backward displacement of water, by the posterior cirri and the consequent swinging of the body about the point of attachment.

It is only after remaining in this position for about 15 minutes that the cyprid moults, and the changes leading to the orientation of organs take place. The sequence of these events is important



II. *Balanus amphitrite varie*

1. Cypris larva just attached. 2. Cypris larva lifted on its antennal stalk at an angle of 40° to the substratum. 3. Cypris after the mouth with deep transverse groove. 4. Cypris larvae after the second rotation effected by the turning inside out of the transverse fold. 5. Animal after absorption of stalk and attachment directly to the substratum. 6. Upper view of animal showing the four deep depressions. 7. Upper view of animal after the formation of the valves. 8a. First pair of thoraic appendage. 8b. Posterior tip of thorax and the abdomen

Ab.—abdomen; Ad.M—adductor muscle; ANT—antennule; AS—anus; Ca—carina; C.E.—Compound eye; Ce.d.—duct of the cement gland; Ce.G.—Cement gland; C.L.—carinolateral valve; M—muscle; Mo.—mouth; N.E.—nauplius eye; O.G.—oil globule; R. L.—Rostrolateral valve; RO.—rostrum; Sc.—Scutum; TH. AP.—thoracic appendages; TH. C.—thoracic cirri

in understanding the mechanics of the first phase of the rotation of the cypris. Pyefinch and Mott (1948) state that "immediately after attachment" the hind end of the cyprid projects upwards. On the otherhand, Darwin (1854), Pagenstecher (1863), and Claus (1869), state that this swinging up takes place "after moulting". Repeated observations have confirmed the author that the first rotation or swinging up of the body of the cypris takes place at the end of the process of attachment, 15 minutes before moulting (i.e. moulting takes place 15 minutes after attachment. This rotation through an acute angle of 40° may be explained as due to two factors (1) the action of the swimming appendages in displacing water backwards and thus lifting the body upwards round the now fixed anterior tip of the antennule and (2) the contraction of the muscles of the antennules straightening the first and second segments and making them come in a line and rotating the body about the point of attachment. It is possible that the 2nd factor is more important.

During the moult, the basal segment of the antennule, the muscles attached to the apodemes, the corium investing the apodemes, and the immediately adjoining parts of the carapace are absorbed and hence a new corium is formed in a deep transverse fold across the whole lower part of the animal (Pl. II. Fig. 3). (Darwin, 1854). The second and third segments of the antennule become fused with their fellows of the opposite side to form a single stalk which remains connected to the outer membrane of the cypris (Pl. II. Figs. 3 & 4). The turning inside out of this deep transverse groove on the ventral side of the body results in a second (internal) rotation of the body of the cypris. The oroanal axis of the cyprid larva already at an angle of 40° to the substratum is now bent at right angles on itself lifting the mouth away from the substratum in such a way that both the anus and the mouth come to occupy an upper position (Pl. II. Fig. 4). The lengthening of the thoraicic feet and their pressure on the thorax and valves probably aid the rotation. During these changes, especially when the mouth is shifted the entire internal organisation of the larva is rotated in such a way that a reversal of surfaces is effected.

Sections taken through the metamorphosing cyprids help us in following the details of the processes resulting in the reversal of surfaces. Since no author has given a complete account of the metamorphosis of sessile barnacles the details of the changes which

constitute metamorphosis in *B.a. variegatus* may be reviewed briefly :

Stage 1 :—is marked by the attachment of the cyprid larva with its ventral surface parallel to the substratum (Pl. II. Fig. 1). In stage 2 the entire body of the cypris is lifted upwards on its antennal stalk before moulting to form an acute angle of 40° with the substratum (Pl. II. Fig. 2). This is effected by the contraction of the muscles (Pl. II. Fig. 2) of the antennules straightening the bend between the first and second segments and making the entire antennule come in a line. In stage 3 the cyprid moults during which the basal segment of the antennule, the muscles attached to the apodomes, the corium investigating the adopomes, and the immediately adjoining parts of the carapace are absorbed as a result of which the new corium is formed in a deep transverse fold across the whole lower part of the animal (Pl. II. Fig. 3). The turning inside out of this deep transverse groove effects the rotation of organs through 90° (Pl. II. Fig. 4).

Note: Comparing these details of the metamorphosis of the sessile barnacle, with Korshelt and Heider's account of the observations made by Claus, Pagenstecher and Darwin on the metamorphosis of stalked barnacles it is found that stage 1 appears to be the same. In stage 2 the cyprid larva is pictured as still remaining parallel to the substratum (Fig. 106 B. page 219). But the oral-anal axis is shown to have undergone a rotation through 90° with the mouth shifted backwards but still remaining ventral and the anus having rotated anticlockwise through 90° and therefore being dorsal in position. These authors explain this rotation as due mainly to the pressure of the developing thoracic feet on the valves. It is not clear how pressure on an external point would result in the rotation of the internal organs especially as the anus and abdomen lie behind the thorax and the tips of the thoracic feet are still shown in a bent condition. In this stage the authors figure the pre-oral fold or the transverse groove (Y of fig. 106 C). In stage 3 the horizontal cypris becomes erected to an upright position (i.e.) at right angles to the substratum. The authors explain this rotation as brought about through the turning inside out of the pre-oral fold. As a result of this erection of the whole body, the oral-anal axis hitherto vertical becomes horizontal once more but with the ventral surface being turned upwards. The dorsal fold of the mantle enclosing the mantle cavity deepens as the result of the rotation (see x. of fig. 106 B). The antennal stalk being bent at right angles at the base (Fig. 106 C) makes one doubt how the turning inside out of the pre-oral groove far behind the point of attachment can effect any rotation through 90° in the region anterior to it and not likely to be affected by the differential growth of the wall in a posterior direction. Stage 4 in which the stalk is absorbed in sessile barnacles is not represented in the Pedunculate barnacles.

This reversion is completed by the absorption of the stalk of the larva and the attachment of the body directly to the substra-

tum. During this stage which constitutes the 4th and final stage of metamorphosis the body which had been rotated through 40°+ 90° is further shifted through 50°. This last phase of the metamorphosis of the cypris takes 7 minutes to be completed and follows the moult of the cypris after attachment (i.e.) the cypris metamorphoses about 22 minutes after attachment. Since the previous authors Darwin (1854), Pagenstecher (1863), Claus (1869), McDougall (1943), Pyefinch (1948[b]) Pyefinch and Mott (1948) and Knight Jones (1953) have not noted the duration of the different phases of metamorphosis, it is difficult to say whether it is accelerated in this form, inhabiting the trophical water. It is probable however that since during the actual process of metamorphosis the larva is neither a cyprid nor a barnacle the transition may have to be as brief as possible in order to secure most chances of survival, and therefore this phase of the life history is likely to be of uniformly short duration in all barnacles tropical or temperate.

Within ten minutes after metamorphosis, the valves of the cypris are thrown off and the body membrane at the sides become thickened and now surrounds the newly metamorphosed larvae. Four deep depressions are formed on this membrane underneath which four calcareous valves make their appearance rapidly by the setting of a gelatinous substance secreted by the body membrane. These four valves are the carina, rostrum, and the paired restro-lateral valves. The alae of the valves have been formed but the radii are not yet discernible. Now the carinal valve splits and gives rise to the paired carino-lateral valves on either side. The scuta and terga also develop by this time from the membrane covering the apex of the body and the adult form is attained.

The duration of the larval stages of *B.a. variegatus* are as follows :

Fertilization of ova to hatching and liberation of 1st nauplius larva	4 days
Duration of 1st nauplius larva	40 minutes
Duration of 2nd nauplius larva	3 hours
Duration of 3rd nauplius larva	5-8 hours
Duration of 4th nauplius larva	4 hours
Duration of 5th nauplius larva	1 hour
Duration of 6th nauplius larva	8 hours
Cyprid larva—Free swimming life	48-72 hours

Process of attachment	15 minutes
Metamorphosis of the cyprid	22 minutes
Formation of valves and compartments	15 minutes

Thus the total time taken by the fertilized egg to attain the adult form, is on an average, 180 hours.

6. *Balanus tintinnabulum tintinnabulum* : This species differs from the previous species in preferring shallow, waters settling only in illumination of low intensity. It reproduces only during July, August and December, January and February. Several observations made in the field show that the ovigerous lamellae contain ova in various stages of development only during these months.

The ripe unfertilized egg is whitish in colour and oval in outline. It measures 0·3 mm. in length and 0·12 to 0·13 mm. in width and is filled with yolk granules. After fertilization the transparent egg becomes opaque. Within two hours after fertilization the white colour changes to light pink and segmentation appears to have progressed. The pink colour persists for nearly ten days at the end of which it changes into light brown and within another four days turns purple in colour and appendages could be distinguished within the fully developed egg. About the 23rd day the embryo shows signs of movement and roughly by the 24th day the egg membranes ruptured and the first nauplius emerges out.

The first nauplius (Pl. III. Fig. 1) is 0·175 mm. to 0·18 mm. at its greatest width. It differs from the larva of *B. amphitrite variegatus* in the carapace being flattened anteriorly with the lateral margins slightly wavy. The abdominal process and the caudal spine are large and more conspicuous than in *B. a. variegatus*. The nauplius eye is situated about 25 μ from the anterior margin. It moults into the second nauplius within 2 hours

The second nauplius (Pl. III. Fig. 2). The carapace has broadened to 0·23 mm. to 0·24 mm. at its greatest width. The frontolateral horns which were directed downwards in the previous stage are now directed upwards even in this stage whereas it is directed at right angles in *B. a. variegatus*. The anterior margin remains flattened and the lateral margin curve slightly downwards posteriorly with a slight protuberance near the posterior end. It moults into the third nauplius within 5 to 6 hours.

The third nauplius (Pl. II. Fig. 3): This nauplius is of stouter build and has a carapace breadth of 0·27 to 0·30 mm. The entire margin is still flattened and the appendages (i.e.) the antennule, the antenna, and the mandible have become conspicuous. The base of the abdominal process unlike in *B.a. variegatus* become swollen even in this stage. This stage lasts for 10 to 15 hours and this period happens to be critical for this barnacle also and more than 70% of the larvae die under laboratory conditions.

The fourth nauplius: (Pl. III. Fig. 4). The carapace measures 0·32 to 0·33 mm. The anterior margin still remains flattened but the lateral margins are convex and taper to the base of the caudal spine. In less than an hour it passes on to the fifth stage.

The fifth nauplius: (Pl. III. Fig. 5). The carapace measures 0·35 mm. to 0·37 mm. As in *B.a. variegatus* the base of the abdominal process now bears six pairs of small spines. The paired eyes make their appearance. Within 8-10 hours it moults into the sixth stage nauplius.

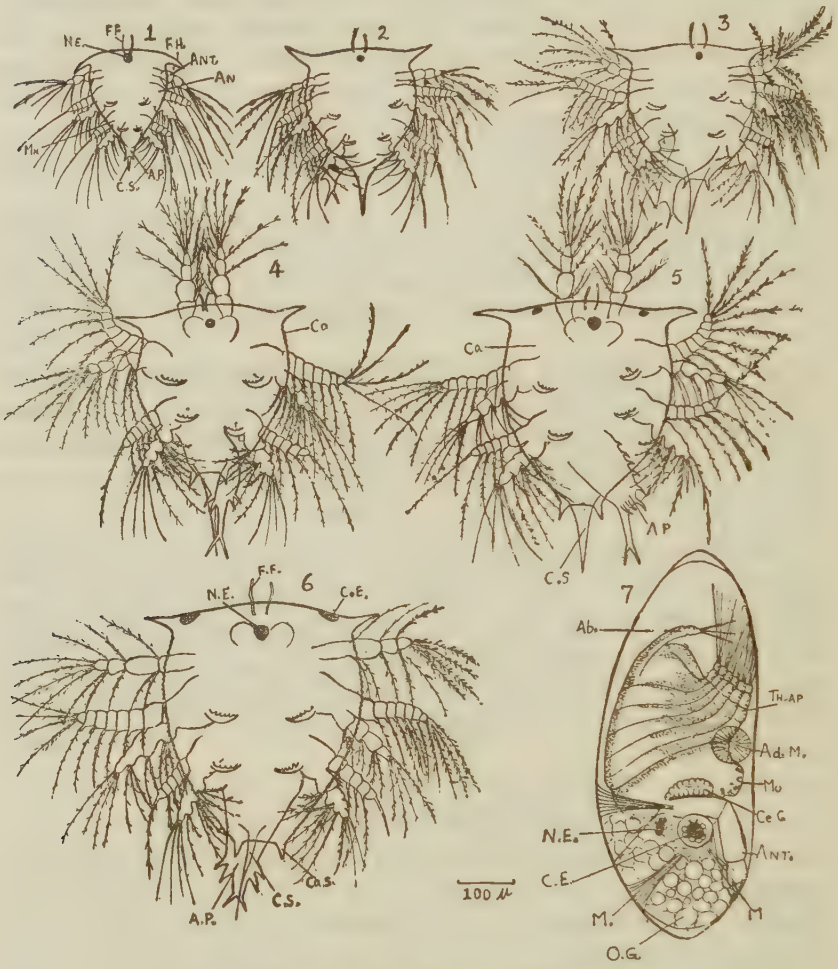
The sixth stage nauplius: (Pl. III. Fig. 6) measures 0·41 mm. 0·42 mm. The anterior margin remains flattened and the larva is longer than broad. The paired eyes are displaced towards the anterior margin and outer sides than in *B.a. variegatus*. It moults into the cypris larva after 12-18 hours. The setation formula of the appendages of the nauplii of this barnacle is given in Table below:

SETATION FORMULA

Stage of Nauplius	Antennule	Antenna	Mandible
1.	0.4.2.1.1	0.1.4-0.3.2.2.2.G	0.1.3-0.3.2.2.2.G
2.	0.4.2.1.1	0.1.6-0.3.2.2.2.G	0.1.3-0.3.2.3.2.G
3.	1.1.4.2.1.1	0.2.6-0.3.2.2.2.G	0.1.3-0.3.2.3.2.G
4.	1.1.4.2.1.2	0.4.6-0.4.3.2.2.G	0.1.4-0.4.2.4.3.G
5.	1.1.1.4.2.1.2.1	0.4.6-0.4.3.2.3.G	0.1.4-0.4.2.4.3.G
6.	1.1.1.4.2.1.2.1	0.4.6-0.4.3.2.3.G	0.1.4-0.4.2.4.3.G

The cypris larva: (Pl. III. Fig. 7). The cypris larva of this subspecies measures 0·82 mm. to 0·89 mm. in length. It leads

a free swimming life for a minimum period of about 72 hours on an average and if conditions are unfavourable for settlement swim



III. *Balanus tintinnabulum tintinnabulum*

FIGS. 1-6. Stages of nauplius of *Balanus tintinnabulum tintinnabulum*
FIG. 7. Cypris larva of *Balanus tintinnabulum tintinnabulum*

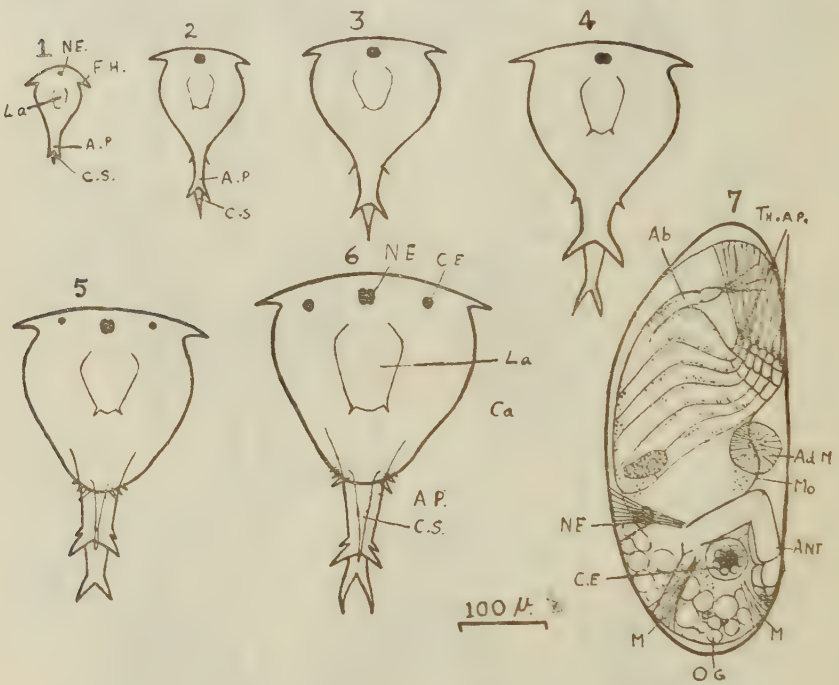
Ab.—Abdomen; A.P.—Abdominal process; An.—Antenna; Ant. Antennule; Ad.M.—Adductor Muscle; Ca.—Carapace; Ca.S.—Carapace Spine; C.S.—Caudal Spine; C.E.—Compound Eye; Ce.G.—Cement Gland; F.H.—Frontal horne; F.F.—Frontal filaments; M.—Muscle; Mo.—Mouth; N.E.—Nauplius Eye; O.G.—Oil Globule; Th.A.—Thoracic appendages

about for a maximum period of 14 days, the mortality rate rising as high as 80% even on the 7th day.

	Madras Form						Plymouth Form Described by Bassindale 1936					
	1	2	3	4	5	6	1	2	3	4	5	6
Time taken for each stage ..	½ hrs.	2 hrs.	4-8 hrs.	4 hrs.	1½ hrs.	5½ hrs.	1 day	1-3 days	4-7 days	1-4 days	1-3 days	1-7 days
Carapace width in mm. ..	0.06-0.08	0.11-0.16	0.16-0.18	0.19-0.20	0.22-0.24	0.27-0.29	.09	.16	.19	.23	.28	.35

The appendages as well as internal organisation are as in the previous species.

The mode of attachment, rotation during metamorphosis and the duration of the two phases of rotation, formation of valves of this barnacle are as in *B.a.variegatus,* described earlier.



IV. *C. S. Stellatus*

FIGS. 1-6. Nauplis of *C. S. stellatus.* FIG. 7. Cypris of *C. S. stellatus*

Abd—Abdomen; A.P.—Abdominal process; Ad.M.—Adductor muscle; Ant.—Antennule; Car.—Carapace spine; C.S.—Caudal spine; CE.—Compound eye; F.H.—Frontal horn; La.—Labrum; M.—Muscle; Mo.—Mouth; N.E.—Nauplius eye.

(C) *Chthamalus stellatus stellatus* :—This barnacle occurs in the supralittoral zone of the rocky shore of Royapuram and is able to tolerate exposure during the low tide periods. It liberates its larvae right throught the year. Field observations on the life histories of several barnacles settled on experimental rock slabs revealed that it attains sexual maturity in about 12 days.

The ripe unfertilized egg is light grey in colour and measures 0·02 mm. Within 40 minutes after fertilization the egg became

yellow and by 24 hours a brownish tinge is obtained. The brownish colour darkens and by the end of the second day it becomes virtually black and roughly 72 hours after fertilization the egg membranes are ruptured and the 1st stage nauplii emerge out.

The development of this species from the 1st stage nauplius to the sixth stage nauplius has been described by Bassindale (1936).

While the general course of development is as described by Bassindale ('36) there is however a slight difference in the size of the larvae. The shortening of the time taken to complete the larval life is a feature worthy of notice and are given in table on p. 41.

Bassindale found that it took 25 days for this species to pass through the six naupliar stages, whereas the Madras form was found to take only 22 hours. Apart from the above mentioned difference, the present form practically agrees with the description and figures given by Bassindale (1936).

For an easy comparison of this subspecies with those of the other two species herein described, the setation formula of the appendages are given in table below and the six different are figured (Pl. IV. Figs. 1-6).

SETATION FORMULA

Stages	Antennule	Antenna	Mandible
1.	0.4.1.1.1	01.4-0.3.2.2.2.G	0.1.3-0.3.2.2.2.G
2.	0.4.2.1.1	0.1.6-0.3.2.2.3.G	0.1.3-0.3.2.3.2.G
3.	1.1.4.2.1.1.	0.1.6-0.3.2.2.4.G	0.1.4-0.3.3.3.3.G
4.	1.1.4.2.2.1.1.	0.2.7-0.5.3.3.4.G	0.1.4-0.4.3.3.3.G
5.	1.1.4.2.2.1.1	0.2.9-0.5.4.4.4.G	0.1.5-0.5.4.3.3.G
6.	1.1.4.2.2.2.1	0.2.10-0.5.4.5-4.G	0.1.5-0.5.4.3.3.G

The sixth stage nauplius larva moults into the cypris larva (Pl. IV. Fig. 2) which measures 0·60-0·63 mm. and lasts for 48 to 72 hours. If conditions are unfavourable, it leads a free swim-

ming life for a maximum period of 16 to 18 days the mortality rate rising high from about the 12th day. The mode of attachment, rotation of organs during metamorphosis and the formation of valves resemble those of *B.a. variegatus* and *B.t. tintinnabulam* described earlier. (vide supra).

III. *Concluding remarks*:—More than the mere morphological differences such as the colour, size, shape of the egg and the setation, relative size of the nauplius and cypris, which it must be admitted, bear out the specific differences vouched for by the adults of the three species, the duration of the embryonic period, the duration of the different naupliar stages, their reactions to different factors of the environment like light, currents, etc., indicate physiological differences due to the different "organism-environment" relation not only of the adult but the larva as well. The fact that these three forms, belonging to different habitats of the same geographical area—Madras—when reared in the same laboratory conditions show these structural and physiological differences in the larval stages and development as a whole, suggest the validity of taxonomic differentiation and descriptions of species. It also suggests that taxonomy can be a more comprehensive science when features of difference in the ecology, physiology and embryology are considered in the recognition and definition of species.

The similarities exhibited by the three different species in the cypris and metamorphosing stages are highly suggestive of the fact that during this period of the lifehistory which is packed with important structural and functional changes, similar physical factors or mechanical exigencies cause or bring about the resemblences in duration, heavy mortality and the pattern of internal changes culminating in the reversal of surfaces. In the same way the three different forms resemble closely in the 3rd naupliar stage being critical and attended by considerable mortality. It is possible that in this period of development also, numerous organisational changes necessitated are crowded together because of the similar physical factors attending development. The structural equipments of the nauplius as well as the cyprids of the three species appear to be of a standard plan permitting but little room for specific divergencies. Similarly the process of attachment, rotation of organs, reversal of surfaces proceed along definite paths fixed and limited by mechanical exigencies allowing but little freedom for diversity of expression.

The similarities as well as the dissimilarities in the development of the three forms would not justify an acceptance of any biogenetic interpretation.

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REFERENCES

Barnes, H. (1953) Size variations in the cyprids of some common barnacles. *J. Mar. biol. Ass. U.K.*, 32: 297-304.

Bate, C. S. (1851) On the development of the Cirripedia. *Ann. nat. Hist.*, 8, Second series, 324.

Bassindale, R. (1936) The developmental stages of three English barnacles. *Balanus balanoides* (Linn), *Chthamalus stellatus* (Poli) and *Verruca stroemia* (*O. F. Muller*). *Proc. zool. Soc. Lond.*, 57-74.

Batham, E. J. (1946) *Pollicipes spinosus* quoy and Gaimard 2. Embryonic and larval development. *Trans. roy. Soc. N.Z.*, 75: 405-18.

Bigelow, M. A. (1896) On the early development of *Lepas fascicularis* *Annat. Anz.*, 263-69.

—— (1901) Notes on the first cleavage of *Lepas*. *Zool. Bull.*, 11: 173-177.

Bohart, R. M. (1929) Observations on the attachment of *Balanus crenatus* Bruguire found in the waters of Puget Sound. *Amer. nat.*, 63: 353-361.

Broch, H. (1927) *Handbuch der Zoologie*. Kukunthal 3. 1 halfe W. de. Gruyeter & Co. Berlin and Leipzig, 503 et. seq.

Burmeister, C. (1843) "Beitrage Zur Naturgeschichte der Rankenfüsser". Muller's Archiv.

Calman, W. T. (1909) *Treatise on Zoology*, Part 7, Appendiculata, Edited by Ray Lankester, 1909.

Claus, C. (1869) Die Cypris-ahnliche Larve (Puppe) der Cirripedien—*Schr. Ges. naturn Marburg*, Suppl. Heft. 5. 17.

Daniel, A. (1954) The Cirripedia of the Madras Coast. *Bull. Madras Govt. Mus. nat. Hist.* 6: 1-42.

Daniel, A. (1955) Studies on the barnacles of Madras. Thesis submitted for the Ph.D. degree of the Madras University (Accepted).

Doochin (1854) The morphology of *Balanus improvisus* Darwin and *Balanus amphitrite niveus* Darwin during initial attachment and metamorphosis. *Bull. Mar. Sci. Gulf. Caribbean.* **1**: 15-39.

Fales, D. E. (1928) The light-receptive organs of certain barnacles. *Biol. Bull. Wood's Hole,* **54**: 534-47.

Filatowa, E. (1902) Quelques remarques a propos du development post-embryonnaire et lanatomic de *B. improvisus. Zool. Anz.,* **25**: 379-385.

Groom, T. T. (1894 a) The life history of the rock barnacle (*Balanus*) *J. Mar. Zool. & Micr.,* **1**: 81-86, **2**: 1-6.

—— (1894 b) On the early development of Cirripedia. *Phil. Trans.* (B), **185**: 119-232.

—— (1895) On the mouth parts of the cypris-stage of *Balanus Quart. J. micr. Sci.* (n.s.), **37**: 269-276.

Groom T. T. and Lock, J. (1890) Der Heliotropismus der Nauplien von *Balanus perforatus* und die periodischen Tiefenwanderungen pelagischer Tiere. *Biol. Zbl.* **10**: 160-177.

Hoek, P. P. C. (1909) The morphology of the later stages of *Balanus crenatus* Brug. *Biol.* Bull. Wood's Hole, **64**: 432-442.

Herz, L. E. (1933) Die cirripedien des nordischen planktons. *Nordisches Plankton,* Bd. 4. No. 8, 265-331.

Jones, L. W. & Crisp, D. J. (1953-54) The larval stages of the Barnacle *Balanus improvisus* Darwin *Proc. zool. Soc. Lond.,* **123**: 765-780.

Knight-Jones, E. W. (1953) Laboratory experiments on gregariousness during setting in *Balanus balanoides* and other barnacles. *J. exp. Biol.,* **30**: 584-598.

Korschelt, E. and Heider, K. (1890-93) Lehrbuch der vergleichenden Entwicklungsgeschichte der Wirbellosen Thiere. 8 vo. Jana. Translated by M. Bernard, edited by M. F. Woodward, as Text-book of the Embryology of Invertebrates, 2. Lond. 1899.

Kunhert, L. (1935) Beitrage zur Entwicklungsgeschichte von *Alcippe lampas* Hancock Z. *Morph.* U. *Okol. Tiere,* **29**: 45-78.

Lochhead, J. H. (1936) On the feeding mechanism of the nauplius of *Balanus perforatus* Burgiere. *Proc. Linn. Soc.* (*Zool.*), **39**: 429-42.

Marshall, S. M. (1925) A survey of clyde Plankton. *Proc. roy. Soc. Edinb.,* **14**: 263-77.

McDougall, K. D. (1943) Sessile Marine Invertebrates North Carolina. *Ecol. Monogr.,* **13**: 321-74.

Nilsson Cantell, C. A. (1921) *Cirripedien Studien. Zool, Bidr. Uppsala,* **7**: 75-378.

Norris, E. & Crips, D. J. (1953-54) The distribution and planktonic stages of the Cirripede *Balanus perforatus* Brug. Proc. *zool. Soc. Lond.,* **123**: 393-409.

Norris, E., Jones, L. W. G., Lovergrove, T., Crisp, D. J. (1951) Variability in larval stages of Cirripedes. *Nature. Lond.,* **167**: 444.

Pagenstecher, A. (1863) Beitrage Zur Anatomic und Entwicklungegeschichte von *Lepas pectinata*. Z. *wiss. Zool.* Bd. **13.**

Plymouth Marine Fauna (1931) Marine Biological Association, Plymouth. 2nd Ed. **27**: 451-582.

Pryor, M. G. M. (1940) On the hardening of the cuticle of insects. *Proc. roy. Soc.*, **91**: 57-65.

Pyefinch, K. A. (1948 a) Methods of identification of the larvae of *Balanus balanoides*, (L), *B. crenatus*. Brug. and *Verruca Stroemia* O. F. Muller. *J. Mar. biol. Ass. U.K.*, **27**: 451-482.

—— (1948 b) Notes on the biology of cirripedes. *J. Mar. biol. Ass.* U.K., **27**: 464-503.

—— (1949) The larval stages of *Balanus crenatus* Bruguiere. *Proc. zool. Soc. Lond.*, **118**: 916-23.

——& Mott, J. C. (1948) The sensitivity of Barnacles and their larvae to copper and mercury. *J. exp. Biol.*, **25**: 276-98.

Runnstorm, S. (1925) Zur Biologie und Entwicklug von *Balanus balanoides* (Linne) *Bergens Mus. Aarbok Naturv. Rackke Nr.* **5.**

—— (1926) Über die platten entwicklung von *verruca stroemia* O. F. Muller Bergens, *Mus. Aarback, Naturv. Rackke*, Nr. 3.

Sandison, E. (1953) The identification of the nauplii of some South African barnacles with notes on their life histories. *Trans. roy. Soc., S. Afr.* **34**: 69-101.

Stubbings, H. G. (1940) Cirripedia (Additional part). *Sci. Rep. Murray Exped.* **7**: 383-99.

Thompson, J. Vaughan (1830) *Zoological Researches*, **1**, 8 vo., Cork, 1830. *Memoir IB.* On the Cirripedes or Barnacles, 69-88.

—— (1835) Discovery of the metamorphosis in the second type of the cirripedes viz., the Lepades, completing the natural history of these singular animals. *Phil. Trans. Part* **2**: 355-58.

Willemoes-Suhm, R. Von (1876) On the development of *Lepas fascicularis* and the Archizoea or Cirripedia. *Phil Trans.* **166**: 182.

Yasugi, R. and Ishida, S. (1937) Free swimming stages of *B. amphitrite albicostatus* *Jap. J. Bot. Zool.*, **5**: 1659-66. **5**: 1659-66.

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Neurosecretion in Crustacea

I. The Neurosecretory Organs of the Eyestalk of *Squilla holoschista**

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ABSTRACT

1. A study of the morphology and histology of the sinus gland and x-organ in *Squilla holoschista* has been made.

2. The sinus gland of *Squilla holoschista* unlike in *Squilla mantis* is situated between lamina ganglionaris and medulla externa. In shape it differs from the latter in resembling both inverse and everse types of gland. In the presence of two distal ventral folds and a dorsal fold the gland appears to be unique.

3. The histological features of the gland are similar to those in other Malacostraca in consisting of very few nuclei and in the absence of cell walls.

4. The x-organ of *Squilla holoschista* differs from that of *Squilla mantis* in being undivided into two parts and situated distal to the medulla terminalis. The cells show varied inclusions found in the cytoplasm which suggest that they represent different stages in the production of the secretory substance.

5. The secretory droplets in the sinus gland of *Squilla holoschista* are of three types which recall the colloids A, B & C described in *Sesarma dahaani* (Enami, 1951). Evidence is cited to suggest a transformation of one type to the other.

Introduction

Following the discovery of the sinus gland in *Paleomonetes vulgaris* (Hanstrom, 1933) a number of workers confirmed its occurrence in other groups of Crustacea (see Sjögren, 1935; Carlson, 1936; Stahl, 1938; Hanstrom, 1939, 1947; Pyle, 1943; Brown, 1944; Passano, 1951 a, b; Enami, 1951; Bliss, 1951; Bliss and Welsh, 1952; Bliss, Durand and Welsh, 1954). Although the gland is essentially a differentiation of the neurilemma covering the optic ganglia

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it appears to differ markedly in structural features varying from a mere disc-like thickening of the neurilemma as seen in *Mysis* to a branched and ramified structure met with in such Decapods, as *Cambarus, Homarus* and *Astacus.* Variations have been noted not only in structure but in the extent and nature of nerve supply. The earlier workers noted only a single nerve arising from the medulla terminalis and supplying the gland but more recent work (Bliss, 1951; Bliss and Welsh, 1952, Passano, 1951 a.b. 52; Carlisle and Passano, 1953; Bliss, Durand and Welsh, 1954) showed, the very complicated nerve supply, one of the main nerves connecting it with x-organ. That the nerve connection between the sinus gland and the x-organ is intimately connected with its functional activity is suggested by the observation of the above mentioned authors, that, contrary to the idea that prevailed up to their time, the sinus gland is not an incretory organ but only a storage place of the secretions produced by the x-organ and transported along the nerve tracts connecting the two organs. Thus a very close parallel has been suggested to the inter-cerebralis cardiacum—allatum system of insects and and hypothalamo—hypo-physeal system of the vertebrates. (Scharrer and Scharrer, 1944). Although the x-organ-sinus gland neurosecretory system is known to have undoubted endocrine function, our knowledge of their structure especially the nature and the extent of variations, is incomplete with reference to many groups. It is felt that a thorough investigation of the morphology and histology of the x-organ and sinus gland is necessary. *Squilla holoschista* has been chosen for this investigation on account of its local abundance and also the fact that very little is known of the sinus gland and x-organ in stomatopoda and therefore a detailed study in the light of the foregoing observation has been made.

ii. *Material and Methods*

Squilla holoschista used in the following study were obtained living from the Madras coast. The observations were mainly based on the serial sections of the eyestalk of the adults in the intermoult period. The sections were prepared from the whole eyestalks from which the exoskeleton was removed. The tissues were fixed in Bouin's or Bouin Duboscq-Brasil and sectioned at 6$^{\mu}$-8$^{\mu}$-10$^{\mu}$ thickness. The stains used were Mallory's triple stain and Heidenhein's iron hemotoxylin. Material double embedded in praffin and celloidin was also used for preparations of sections. Sections of the eyestalk were also prepared by the freezing method.

For this purpose the eyestalks were fixed in 5 % saline formalin for 12-24 hrs. and infiltrated through 12½% and 25% gelatin successively and blocks were made using paper boats. Sections were cut 20-25$^{\mu}$ thick. Such were stained with 2 % methylene blue (Welsh, 1941) and cleared with glycerine. Alternate staining and clearing gave good results.

The Sinus Gland (Fig. 1-S.GL.)

The sinus gland of *Squilla mantis* has been described in some detail by Hanstrom (1937, 1947) who pointed out that it belongs to the inverse type of gland found in a number of Decapods. He also made a reference to the structure of the gland in *Squilla desmaresti.* From the figure of the eyestalk in *Squilla mantis* given by Carlisle and Passano (1953) it may be inferred that the position of the sinus gland with reference to the optic ganglia may vary. Hanstrom (1939) described that the gland is situated between the medulla externa and medulla interna but in the figure given by Carlisle and Passano (1953) it is shown to occupy a position distal to lamina ganglionaris. Very little is known of the structural features and histological details of the sinus gland in Stomatopoda.

From the examination of sections of the eyestalk of *Squilla holoschista* it is seen that the sinus gland lies on the dorsal side of the optic ganglia between the lamina ganglionaris and medulla externa over which it extends proximally (Fig. 1, S. Gl.). The position of the gland appears to differ from that in the allied species *Squilla mantis* and probably this is attributed to the peculiar arrangement of the ganglia in the eye-stalk of *Squilla holoschista.* Unlike in Decapoda in which they are found in vertical series one behind the other, here the ganglia are not so regularly arranged as the medulla interna appears to be displaced to one side. As in *Squilla mantis* the gland is completely separated from the neurilemma covering the optic ganglia and unlike in *Squilla desmaresti* in which the gland is partly connected with the neurilemma. The gland is triangular in shape and measures about 0·5 mm. in length and about 0·65 mm. across the broadest part. It is curved with the inner side in contact with the portions of the optic ganglia (Fig. 9. S.GL.). At the anterior extremity of the gland it is bent on itself to form two well marked folds. Such folds have not been described in any other crustacean and appears to be peculiar to this type. These folds lie on each side of the

median logitudinal plane of the gland (Fig. 4). A blood vessel passes inbetween the folds. The extensions of the folds enclose the blood sinus partially. In some of the larger sized glands the fold on the ventral side possesses small extensions of the tissue protruding into the blood sinus. The gland is surrounded by a large outer blood sinus, and a smaller inner sinus which appears to be in the form of a blood vessel already referred to, occurs between and partially enclosed by the folds of the gland. The nerve supplying the gland enters on its inner side which is concave. The structureless membrane or the limiting membrane is found covering the gland on the convex side and is absent on the inner concave side recalling the condition described by Hanstrom (1939) in Anomura. From the above descriptions it is seen that the gland found in *Squilla holoschista* does not conform to either the typical inverse type or the everse type. Although it is not so complex as the highly branched corrugated types seen in *Cambarus, Homarus* and *Astacus* it is not so simple as that of Mysidacea in that it is completely separated from the surrounding ganglia cells and in being distally folded, features which may be considered as indicating a more advanced condition. In these respects the gland differs from that in the allied species *Squilla mantis* as described by Hanstrom (1939). By possessing the structureless membrane on the outside and the nerves on the inside, it resembles the gland found in the Anomura (Hanstrom, 1939) but differs from it in being situated within the eyestalk and in possessing an elongated folded appearance. Although in the distal folded region the structureless membrane lines a small strip of space which is open above, it differs from the typical inverse type of the sinus gland described in *Acanthephyra* or *Callinectes*. It is clear that the sinus gland of *Squilla holoschista* does not conform to any of the previously described types.

In sections stained with iron hematoxylin the sinus gland shows the presence of very few nuclei which are found scattered (Pl. 1, Nu.). The sinus gland tissue has numerous radially arranged tubules which are filled with many aniline blue-stained secretory droplets. In more recent work these are considered to be the nerve endings (Bliss, 1951; Bliss and Welsh, 1952; Passano 1951 a & b, 1952; Bliss, Durand and Welsh, 1954). Formerly they were thought to be secretory tubules by Hanstrom (1939, 1947) and his collaborators (Sjögron, 1934; Stahl, 1938; Carlson, 1936, Brown, 1944). The secretory droplets in animals in the middle

inter-moult stages are stained intensely with aniline blue but show marked variation in the intensity of staining. Those that stain intensely are round or oval in shape. In between them are found the less intensely staining ones which are usually elongated in shape. Another type of secretory droplet which is very lightly stained with aniline blue is irregular in shape and has a granulated texture. Fig. 7 (SD1, SD2, SD3) shows the three types of secretory droplets referred to above. The distinction between the first type and other two types of secretory droplets are clearly seen in the figure. Scattered among the anastomosing nerve fibres on the ventral concave side of the sinus gland are some bulbous nerve endings filled with small oval shaped acid fuchsin stained granules which are arranged in concentric rows, (Fig. 8a). Some of them have been found in certain specimens to stain very lightly with aniline blue. From the observations that these granules found in the bulbous nerve endings resemble markedly those occurring in the sinus gland, it may be suggested that the secretory droplets found in the nerve endings which are acid-funchsinophil change into light aniline blue stained droplets and these later form the three types of droplets described in the sinus gland. From an examination of number of sections, the intermediate stage in such transformation can be made out. The small oval shaped granules found at the bulbous nerve endings are similar to those occurring in the large granulated masses described in the sinus gland which are very lightly stained with aniline blue. This possibly represents the first type of secretory droplets which undergo transformation. The change from this to what may be called the second type which is elongated and still lightly stained with aniline blue is suggested by a comparison of the above mentioned two types of droplets. The oval shaped, intensely aniline blue-stained droplets appear to have been derived from the second type mentioned above. In *Sesarma* (Enami, 1951) described similarly three types of secretory droplets in the sinus gland which he called colloid A, B and C. The colloid A constitute ovoid or ellipsoidal masses staining intensely with acid fuchsin of the Mallory stain. The droplets forming what is called colloid B are irregularly shaped apparently formed by a coalescence of masses of colloid A. These droplets stain in a feeble way with acid-fuchsin and may include a number of granules which stain with aniline blue. The colloid C consists of shapeless masses which grew to large size possibly by coalescence with other material and stain with aniline blue and other basic stains. A number of vacuoles occur in it, containing inclu-

sions of various kinds. It has been suggested that the three types of colloid show a continuity in that colloid A is suggested to be transformed into colloid C by way of colloid B and that Colloid A is derived from the nuclei occurring in the sinus gland. A comparison of the above features with what has been observed in *Squilla holoschista* shows that the secretory droplets in question are not derived from the nuclei as suggested by Enami but from the bulbous nerve endings. This is so convincingly seen in Fig. 8a in which the inclusions in the bulbous nerve endings in the sinus gland and the secretory droplets described above are seen. Both structural features and staining reactions suggest their derivation from the inclusions in the nerve endings. Further the nuclei of the sinus gland are seen at this stage and their features do not suggest any activity which may be related to the formation of these secretory droplets. In this connection it is of interest to point out the observations of Bliss & Welsh (1952) that, what Enami described as the capsule of the nucleus which surrounds colloid A and which he adduced as an evidence to support his view that these colloids are secreted by the nuclei of the sinus gland, are really not such, but are only the enlarged nerve endings which enclose the colloid in question.

The innervation of the sinus gland in Stomatopoda has so far not been studied in detail. Hanstrom described a single nerve arising from the medulla terminalis which has been shown later (Bliss, 1951, Bliss and Welsh, 1952; Passano 1951 a & b, 1952; Carlisle and Passano, 1953; Bliss, Durand and Welsh, 1954) to originate from the x-organ as seen in *Gecarcinus* and other Decapoda. As noted by the later authors, it is seen from the examination of the sections of the eyestalk of *Squilla holoschista*, that the main nerve innervating the sinus gland is the one arising from the x-organ. This nerve is thick and coarse and lies in the centre of the x-organ (Fig. 10-x-ORN).. From each lobe of the x-organ the axons of each x-cell form a small bundle of nerve fibre which meet in the centre of the organ to make up the x-organ nerve. This nerve leaves at the proximal end of the x-organ and descends downwards to the peduncle lobus opticus (Fig. 10-x-ORN). It then curves round the peduncle and ascends upwards through a space between the ganglia and the neurilemma and breaks up into individual fibres on the inner concave side of the sinus gland. The tips of these fibres are bulbous as has been noted in *Gecarinus lateralis* (Bliss and Welsh, 1952). Another nerve which supplies

the sinus gland comes from the peduncle lobus opticus. A similar nerve was first noted in *Cambarus bartoni* by Welsh (1941) who used methylene blue technique to trace its course. The origin of this nerve has been recently traced by Bliss, Durand and Welsh (1954) to the groups of neurosecretory cells situated in the brain. This nerve enters the sinus gland and contributes a part of the gland tissue on the lateral sides. In addition to the nerves described above a large bundle of nerve fibres arising from the dorsal side of the medulla terminalis join the x-organ nerve before its entry into the sinus gland. These nerve fibres as in *Gecarcinus* and *Cambarus* (Bliss, Durand and Welsh, 1954) appear to arise from the neurosecretory cells occurring in the medulla terminalis.

The x-organ

Our knowledge of the x-organ in Stomatopoda is based on the account given by Hanstrom (1931, 1934) from a study of *Squilla mantis*. The x-organ is of the compact type found in most Decapods and located in the brain and formed of vesicular nuclei which recall those of the ganglion cells. It was also noted that the organ is unlike the hollow type found in Mysidacea. From more recent studies on the structure and histology of the x-organ by Carlisle and Passano (1953) it is known that in Stomatopoda, as in Natantia, the organ is divided into two halves which are connected by a nerve and situated on the proximal ventrolateral corner of the medulla terminalis. In the present investigation the x-organ lies on the lateral side in a depression formed by the medulla externa, medulla terminalis and the displaced medulla interna (Fig. 1 X. Or., Pl. 2, X. Or., Fig. 10-X-O8). In being situated distal to the medulla terminalis it resembles the x-organ of *Homarus americanus* (Pyle, 1943) and *Palaemonetes vulgaris* (Hanstrom, 1938) and differs from that of *Squilla mantis* as well as those of *Sesarma dahaani* (Enami, 1951), *Cambarus bartoni* (Welsh, 1941) and many species of Brachyura. The x-organ extends on the medulla terminalis from the ventral side to the dorsal side in an oblique manner (Fig. 10-X. Or.), and is undivided unlike in *Squilla mantis* (Carlisle and Passano, 1953). The only indication of a division, if any, is a light construction in the distal region which gives it a lobed appearance. It has the characteristic shape of a cluster of grapes surrounding the thick nerve which descends through the *medulla terminalis* recalling the structure found in Natantia (Hanstrom, 1939).

The histology of the x-organ in *Squilla mantis* is not known in detail but it has been described in *Palaemonetes vulgaris* and other Decapods. In *Palaemonetes* Hanstrom (1939) found several x-cells in each cluster whose nuclei are round resembling those of the ganglion cells but differing from the latter in the presence of secretory droplets. In *Homarus americanus* (Pyle, 1943) each cluster or unit of the x-organ is composed of 20 or more cells with their nuclei arranged in the periphery with secretory droplets in the central region and supplied with nerves. Similar histological features are seen in *Squilla holoschista* although the number of cells in each cluster may be much less. Carlisle and Passano (1953) have described different types of cells occurring in the x-organ in Brachyura and Natania but very few details have been given regarding Stomatopoda. In *Squilla holoschista* the position of the nucleus and the number and size of the secretory droplets vary in the x-cells. On this basis a number of types of x-cells could be made out. The cells (Fig. 11 and 12-Ns) which are found in large numbers in the distal half and less so in the proximal half of the gland are triangular, tetragonal or oval in shape with one of the apices produced into the neuron. The cells are rich in cytoplasm each with a rounded nucleus and without vacuoles. A marked resemblance is noted to the giant β neurosecretory cells described in the Brachyura (Carlisle and Passano, 1953) but differs from them in being smaller and in not possessing secretory droplets within the cell-body or the axon. Another type (Fig. 13) of cells occurring more in the prominal half is spherical in shape in which the nucleus is round, similar in structure to those of the first type and found in the centre of the cell. In cytoplasm are found small spherical acid-fuchsin staining secretory droplets. The third type (Fig. 14) of cells which alsc occur in the proximal part are numerous and the nuclei are situated on the periphery of the cells while the intensely staining large secretory droplets occupy the central region of the cells. The cells are often vacuolated and sometimes the perinuclear space is filled with a single large inclusion. This type of cell resembles the so-called second type of cell mentioned by Carlisle and Passano (1953) in the Brachyura. In addition to these there are irregular secretory droplets, similar in staining reaction to the inclusions described above which occur in a large space in the proximal part of the gland (Fig. 15). These are possibly set free by the breaking up of the secretory cells. At this stage the occurrence of a few nuclei in the proximity of the bodies lend support to this

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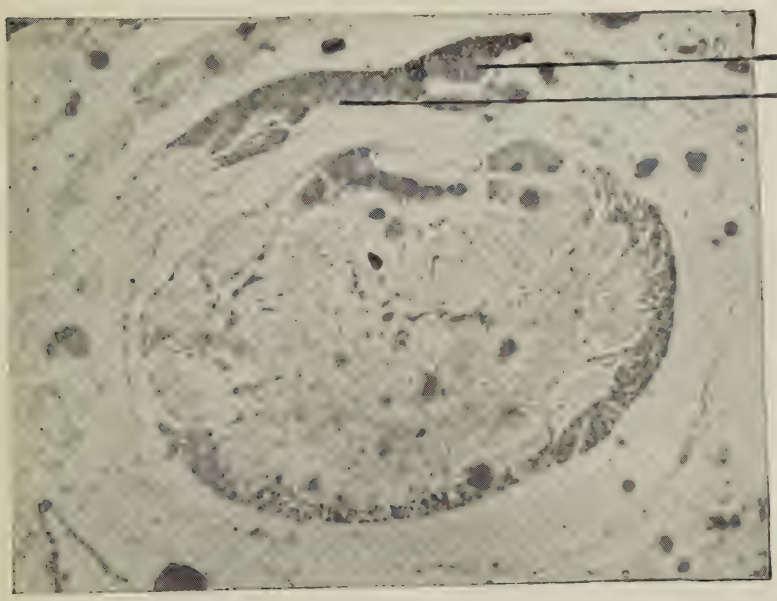
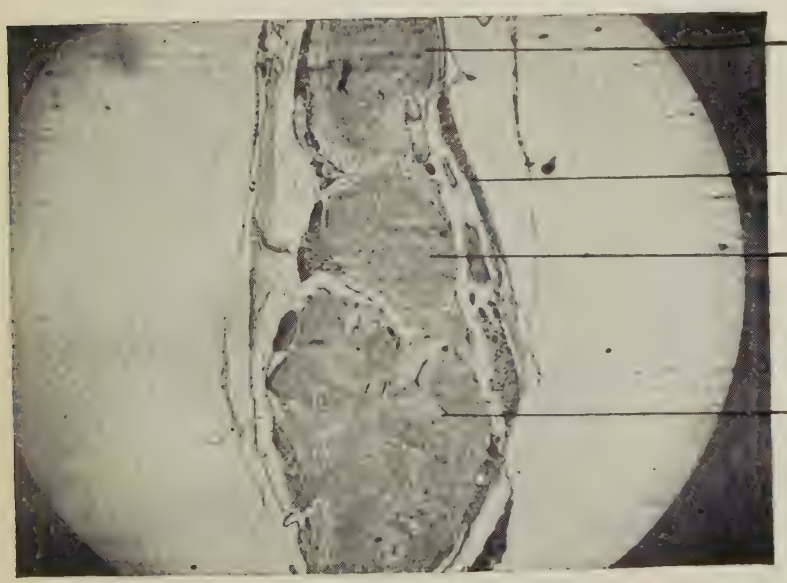


1

T.S. of the Sinus Gland

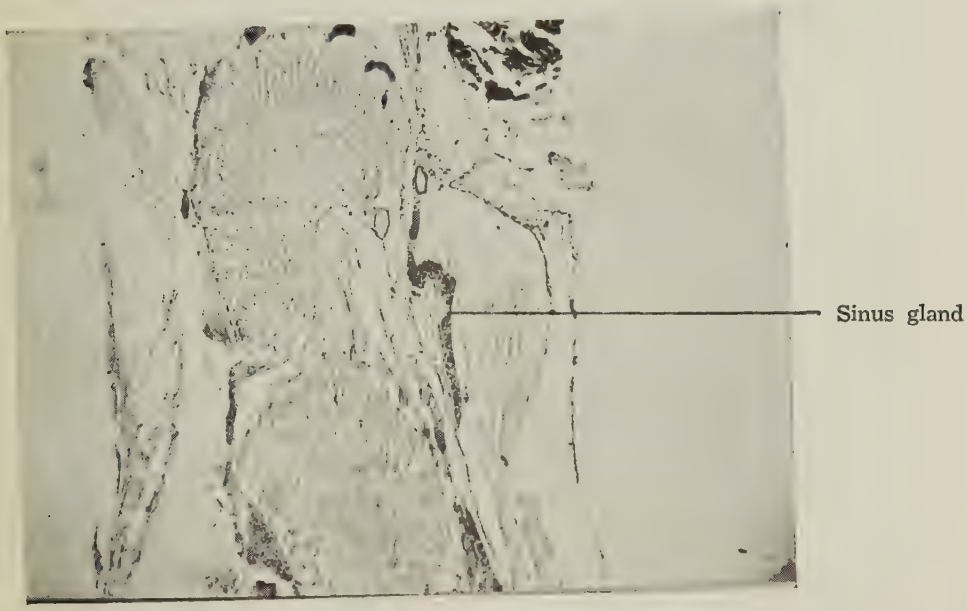


2

L.S. of the Sinus Gland
(Young Specimen)

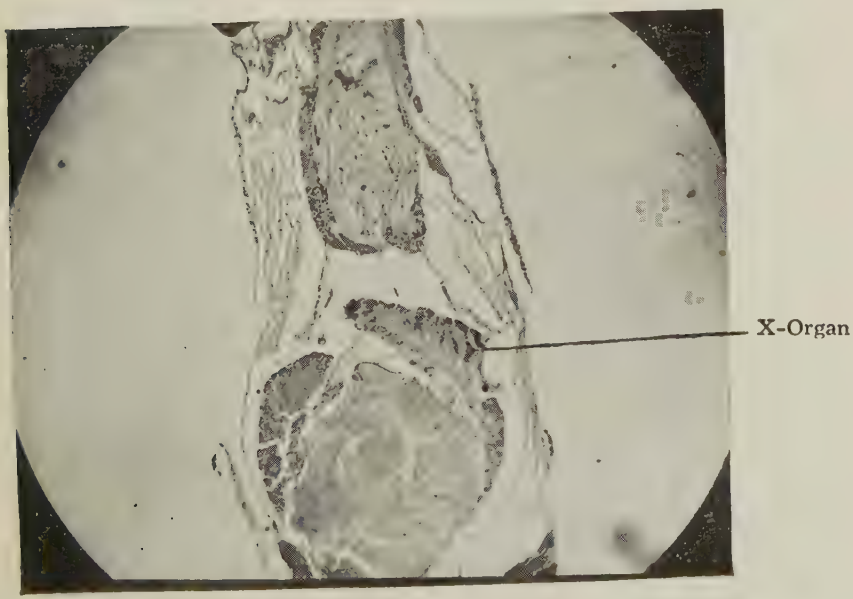
RUBY DANIEL

SQUILLA HOLOCHISTA



3

L.S. of the Sinus Gland
(Young Specimen)



4

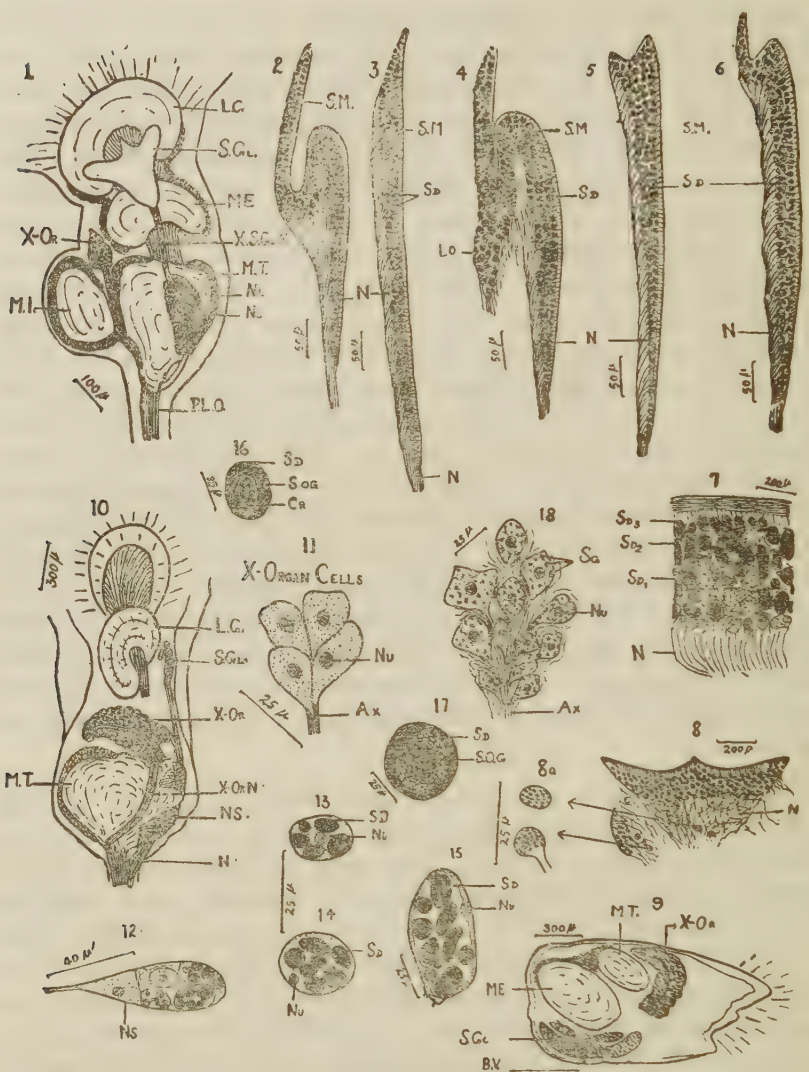
L.S. of the X-Organ

RUBY DANIEL

assumption that the inclusions mentioned above may have been derived from the breaking up of the secretory cells which contained them. These observations recall strongly what has been described as the third cell type in the x-organ of Brachyura (Carlisle and Passano, 1953). The absence of a nucleus in the so called cell type led the authors to regard these as products of secretion rather than cells. The secretory droplet shows one or two concentric rings and sometimes a very minute clear space is seen in the middle of the innermost ring (Fig. 16). These are found near the place where the nerve leaves the x-organ. In the larger droplets with concentric rings (Fig. 16-CR) there occur in the outer region a few very small shining oval shaped globules arranged in a concentric manner. In still larger ones such oval shaped globlues are very numerous in number (Fig. 17). From the above observations it may be presumed that the largest secretory droplet with numerous oval-shaped globules represent the end stage in the development of the secretory droplets of the x-organ. Since similar oval shaped acid fuchsin staining granules are found in the bulbous x-organ nerve endings near the sinus gland, it may be inferred that the largest secretory droplet in the x-organ breaks up into minute oval-shaped granules and in this manner the secretions appear to pass through the nerve fibres.

Histologically it can be seen that there is a graded development of the cells ranging from those which do not show any evidence of secretory activity to those with variously sized secretory droplets leading on to the large irregular droplets which have the oval-shaped globules (Figs. 16 & 17). Carlisle and Passano (1953) in their descriptions of the x-organ in Brachyura considered the cells they found as constituting different types. From the foregoing account of the x-organ cells in *Squilla holoschista* it appears reasonable to suggest that the different cell types found are all of the same kind and represent different stages in the development of the secretory cells with inclusions in the cytoplasm. It seems likely that these inclusions are liberated with the breaking up of the cells so as to form the large secretory droplets described above.

Whether the x-organ is supplied with a nerve connection from any of the optic ganglia is not known for certain. The earlier authors (see Hanstrom, 1939) considered that the x-organ is supplied by a nerve from the medulla terminalis but later work has shown that the nerve mentioned by Hanstrom and others does not



I. *Squilla holoschista*

1. Dorsal view of the eyestalk. 2. Section passing through the fold region (in young specimen). 3. Section passing passing through the median plane of the gland. 4. Section passing through the fold region (in mature specimen). 5. Section of the sinus gland in specimens measuring 7 cm. in length. 6. Section passing through the fold region (in young specimen). 7. Transverse section of the sinus gland. 8. Longitudinal section of the sinus gland. 8a. Bulbous nerve endings. 9. Cross section (slightly oblique) of the eyestalk. 10. Sagittal section of the eyestalk. 11. X-organ neurosecretory cells. 12. A lobe of the X-organ. 13. X-organ cell with the nucleus in the centre . 14. X-organ cell, with the nucleus in the periphery. 15. A large space having secretory droplets. 16. Secretory drop-

originate in the medulla terminalis but in the x-organ (Passano 1951 a & b, 1952; Bliss, 1951, Bliss & Welsh, 1952, Carlisle & Passano, 1953; Bliss, Durand & Welsh, 1954). From the observations made in the course of this study it is apparent that there is no special nerve supply from the optic ganglia to the x-organ. As in the Decapods studied by the above mentioned authors a bundle of nerve fibres connect the cells of each unit of the x-organ and these join together to form the large tract which has been shown to pass to the sinus gland.

In addition to the neurosecretary cells found in the x-organ described above, there is an extensive group of cells showing evidence of secretory activity situated in the dorsal aspect of the medulla terminalis. (Fig. 1 & 18 Ns). In position these cells correspond to the group named E2 in *Gecarcinus* and *Cambarus* (Bliss, Durand and Welsh, 1954). There are no other secretory cell groups in the eyestalk of *Squilla holoschista* to compare with the groups E3, E4 and E5 mentioned by the above mentioned authors. The cells which may be said to correspond to the E2 agree in shape, structure and staining reactions with those described in the forms mentioned above.

Discussion

It is seen that the sinus gland presents a variety of form and structure not only in different groups of Malacostraca so far studied but even within the limits of a genus as seen in *Squilla*. In *Squilla mantis* (Hanstrom, 1937) the gland is known to conform to what is called a typical inverse type but from the foregoing account it is clear that in the allied *Squilla holoschista* the gland is different in structure in not conforming either to the inverse or the everse type (see Hanstrom, 1939) and in the absence of a free central cavity in the gland. However, in some features the gland recalls the everse type described in the Anomura such as

let showing a concentric ring and oval shaped globules. 18. Neurosecretory cells found near the medulla terminalis.

Ax—Axon; B.V.—Blood vessel; C. R.—Concentric ring; L.G.—Lamina ganglionaris; LO.—Lobe; ME.—Medulla externa; M.I.—Medulla internal M.T.—Medulla terminalis; N.—Nerve; N.L.—Neurilemma; NS.—Neurosecretory cells; Nu.—Nucleus; P.L.O.—Peduncle lobus opticus; S.D.—Secretory droplet; S.GL.—Sinus gland; S.M.—Structureless membrane; S.O.G.—Shining oval globules; X-Or.—X-organ; X-orN—X-Organ nerve; X-S.GLN—X-organ sinus gland nerve

in the occurrence of the structureless membrane on the periphery and in the entry of the nerves on the inner side of the gland. The features unique to this species are the presence of two distal folds occurring on either side of the median plane and the presence of lobes on the folds. The x-organ as in the case of sinus gland presents a marked variability in its shape and structure. In *Squilla holoschista* the x-organ presents resemblances to that of *Homarus* in being lobed and separated from the surrounding ganglion cells by a thin connective tissue sheath. Its characteristic appearance as cluster of grapes recalls strongly the condition of the x-organ in Natantia (Hanstrom, 1939). It is seen that the morphology of the x-organ presents wide variation in the crustaceans ranging from a mere cluster of nerve cells to a well organised structure presenting definite features. That variations in structure occur even in closely allied forms is suggested by the observations that in *Squilla mantis* (Carlisle and Passano, 1953) unlike in the present form, it is divided into two parts and is associated with the sensory pore which as pointed out by Hanstrom (1939) is a primitive character. By comparing with it, the x-organ of *Squilla holoschista* shows more advanced features and resembles in a number of respects that found in *Homarus*.

Till recently the sinus gland was considered an incretory organ and the secretory droplets found in it to be products of its own secretion, but it has been noted even by the earlier workers that only very few nuclei could be made out in the gland and that the cell-walls were completely absent. Pyle (1943) thought whether the sinus gland may be only a storage place for the secretions produced elsewhere. That this is so, has been proved in *Gecarcinus lateralis* and other Decapods in which Bliss (1951). Passano (1951 a & b, 1952), Welsh (1951), Bliss & Welsh (1952), Carlisle and Passano (1953), and Bliss, Durand and Welsh (1954) have shown that the sinus gland receives the secretions from various neurosecretory centres and transported along the nerve tracts. The observations made on the sinus gland of *Squilla holoschista* abundantly support the above view not only by the presence of secretory droplets in the nerve tracts supplying the organ which are similar to those occurring in the gland, but also by the structure of the gland which, as already observed by the above mentioned authors in the Decapods is formed by the nerve fibre endings containing various secretory droplets.

In *Squilla holoschista* there is no evidence of local secretory activity. The secretory droplets found in the sinus gland of *Squilla*

holoschista recall the so-called colloids A. B. & C. described in the sinus gland of *Sesarma* (Enami 1951) and suggest that as in the latter the different types of inclusions are formed by the transformation of one type into the other. But in *Squilla holoschista* it is clear that these inclusions are not produced by the secretory activity of the nuclei present in the sinus gland as suggested in *Sesarma* by the above author but are transported to it along the nerves supplying it. That the source of the secretions which are found in the sinus gland is mainly from the x-organ is suggested from the evidence adduced in regard to the similarity of the inclusions in the sinus gland to those found in the x-organ and from the occurrence of similar inclusions along the nerve supplying the sinus gland. It might be suggetsed that in *Squilla holoschista* as in the Decapods there is a passage of the secretory matter from the x-organ to the sinus gland which together form a single neurosecretory system.

The intervention of the sinus gland which seems to be intimately related to its functional activity was thought to be very simple by Hanstrom and his collaborators but recent work has emphasized its complexity (Bliss, 1951; Welsh, 1951; Passano, 1951, 1952; Bliss & Welsh, 1952; Carlisle and Passano, 1953; Bliss, Durand & Welsh, 1954). In *Squilla holoschista* the nerve supply recalls the condition noted in *Gecarsinus* and *Cambarus virilis*. Although the origin of the nerve coming from the peduncle of the lobus opticus has not been traced, it is known that a nerve corresponding to this in *Gecarsinus* and *Cambarus* has been traced to the neurosecretory cells situated in the brain and probably in the thoracic ganglia (Bliss, Durand & Welsh, 1954). The main nerve supplying it is formed from the axons of the x-cells and this establishes a connection between the x-organ and the sinus gland and shows the presence of secretions at the extremities. These features are suggestive of one of the main functions of the nerve supply. The other nerves supplying the organ have also been shown to originate from other neurosecretory centres and serve a similar function. Welsh (1941) suggested that the nerves supplying the sinus gland may play a part in the regulating of the activity of the gland by transmission of nerve impulses.

Bliss (1951), Passano (1951, 1952), Welsh (1951), Bliss & Welsh (1952) and Bliss, Durand and Welsh (1954) from their work in the structure and functions of the sinus gland and x-organ pointed out that they are not separate individual endocrine organs but that they together with other groups of neurosecretory cells

of the brain form a single complex neurosecretory system. The study of the sinus gland and x-organ in *Squilla holoschista* lend support to the idea put forth by the above mentioned authors. Further, as suggested by them in structural features and the probable mode of functioning, a close parallel may be noted to the intercerebralis-cardiacum-allatum system in insects and the hypothalamo-hypophysical system in vertebrates.

Acknowledgements

The author wishes to take this opportunity to express her thanks and gratitude to Dr. G. Krishnan, M.A., M.Sc., Ph.D. (Manchester), Reader, Zoological Research Laboratory, University of Madras, for suggesting this problem and for his unfailing guidance throughout the course of this investigation. She is also thankful to Prof. C. P. Gnanamuthu, M.A., D.Sc., F.A.Sc., F.Z.S., Director, Zoology Research Laboratory, University of Madras for going through the manuscript and offering suggestions. Thanks are also due to the University of Madras for the award of a studentship during the tenure of which the work was carried out, and to the Ministry of Education, Government of India for awarding a Senior Research Scholarship during the tenure of which this paper was written.

REFERENCES

Alikunhi K. H. & Aiyer R. G. (1948) "Growth in Stomatopoda" *Curr. Sci.*, **12**: 80.

Bliss, D. E. (1951) "Metabolic effects of the sinus gland or eyestalk removal in the land crab. *Gecarcinus lateralis*". *Anat. Rec.*, **111**, 502.

——& Welsh, J. E. (1952) "The neurosecretary system of Brachyuran Crustacea", *Biol. Bull., Wood's Hole*, **103**: 157.

—— D.E., Durand, J. B. & Welsh, J.H. (1954) "Neurosecretary system in Decapod Crustacea", Z., *Zellforsch Bd.* **39**: S. 520.

Brown, F. A. (1944) "Hormones in Crustacea. Their sources and activities". *Quart. Rev. Biol.*, **19**: 32-46-118.

Carlisle, D. B. (1953) "Moulting Hormones in Leander" *J. Mar. biol. Ass.* U.K., **32**: (2), 289.

—— & Passano, L.M. (1953) "The x-organ of Crustacea". *Nature, Lond.*, **171**: 1070.

Carlson, S. P. (1936) "Colour changes in Brachyuran Crustaceans, especially *Uca* pugilator", *Kurgl. Fysiogr. Sallsk. Frohandl. Lurd*, **6**: 63.

Enami, M. (1951) "The sources and activities of two chromatophortropic hormones in crabs of the genus *Sesarma*. II. Histology of incretory elements". *Biol. Bull., Wood's Hole*, **101**: 241.

Frost, R., Saloum, R. & Kleinhol, L. H. (1951) "Effect of sinus gland and of eyestalk removal on rate of O_2 consumption in *Astacus*". *Anat Rec.*, 111: 572.

Hanstrom, B. (1931) "Nerve Untersuchungen uber sinnes-organs and Nerven system der Crustacean", l. Z. *Morph. Okol, Teire,* 23.

—— (1933) "Neue untersuchungen uber sinnessorgane and Nerven system der Crustaceen" II. *Zool. Jahrb.* Abt. f. *Anat.*, 56: 387.

—— (1937) "Die Sinusdruse and der hormoral bedingte Farbwechsel der Crustaceen" Kungl. *Svenska Vetensk.*: Nr. 3 *Handl.* 16 Akad.

—— (1939) "Hormones in Invertebrates" Oxford. At the Clarendon Press.

—— (1947a) "The three principal incretory organs" in the animal kingdom. The sinus gland in Crustacea, the Corpus-cardiacum-allatum in insects, the hypophysis in Vertebrates". Three lectures at the University of London, 1946.

Oelze, C. (1931) "Beitrage zur Aratomie von *Diastytis rhatkei*". *Zool. Jahrb., Abt. f. Anat.*, 54.

Passano, L. M. (1951a) "The x-organ sinus gland neuro-secretory system in crabs", *Anat. Rec.*, 111: 502.

—— (1951b) "The x-organ, a neurosecretory gland controlling moulting in crabs. *Anat. Rec.*, 111: 559.

—— (1952a) "Phase contrast observations on living neurosecretory cells of *Sesarma*". *Anat. Rec.*, 112: 460.

Pyle, R. W. (1943) "The histogenesis and cyclic phenomena of the sinus gland and x-organ in Crustacea". *Biol.* Bull., *Wood's Hole,* 85: 87.

Scharrer, B. & Scharrer, E. (1944) "Neurosecretion. IV.A. Comparison between the intercerebralis qartiacum allatum system of the insects and the hypothalamo hypophyseal system of vertebrates". *Biol. Bull., Wood's Hole,* 87: 242.

Sjogren, S. (1934) "Die Blutdruse and ihre Assbildurg bei den Den Decapoden". *Zool, Jahrb. Abt. F. Anat.*, 58:145.

Stahl, F. (1938) "Preliminary Report on the colour changes and the Incretory Organs in the Heads of some Crustaceans", *Ark Zool:* Stockholm, 30 B: No. 8.

—— (1938a) "Uber das Vorkommen von inkretorischen Organan und Farbwechselhormonem in Kopf eiginer Crustacean", K. Fysiogr. Sällsk. Lund Förr.. 49 No. 12.

Travis, D. F. (1951) "The control of the sinus glands over certain aspects of calcium metabolism in *Panulirus argus* Latreille". *Anat. Rec.*, 111: 503.

Welsh, J. H. (1941) "The sinus gland and 24 hour cycle of pigment migration in the crayfish". *J. exp. Zool,* 86: 35.

—— (1951) "New evidence concerning the source and action of the eyestalk hormone". *Anat, Rec.*, 111: 442.

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Green Colour Preservation of Plants

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ABSTRACT

The available methods of preservation of green colour of plants in fluids are discussed, and attention has been focussed on developing a cheap, simple and effective method. The procedure suggested, requires the employment of two solutions—Solution A, saturated solution of copper sulphate in 10% aqueous formalin; and solution B, 5% aqueous formalin. The specimens treated with these two solutions have retained their original colour for *ten* years without undergoing any kind of deterioration.

Introduction

Artificial preservation of the natural colours in killed or preserved plant materials, has received rather sporadic attention of the biologists in general. It has a special advantage in museum galleries, and class room instructions (where attractive and impressive specimens are needed), over the other general preservation methods that subordinate the retention of original colours to micro-technical needs. As long ago as 1838, Cleghorn (1838) has suggested preserving the given specimen *with* colour in a saturated solution of common salt in water. This method preserves the colour only for a few months; and traces of salt get deposited as dirt on the inner sides of the container. Butler (1918) suggests adding citric acid to 1% solution of sodium bisulphite in which the given specimen is kept immersed, till a strong odour of sulphur dioxide is given off; the specimen is then transferred to a 4 % formalin. The result is however not satisfactory so far as the preservation of the green colour is concerned. Matthews (1920), Bishop (1932) and Rowley (1943) have recommended external application of coal-tar and other dyes on dried plant specimens. This is suitable for preparing exhibits in natural history museums where the emphasis is laid more on the zoological exhibits than on the botanical ones. But, owing to the shrinkage and distortion that the specimens suffer on drying, and owing to the un-

satisfactory nature of the coal-tar dyes in the tropics, this method should be unacceptable to institutions where Botany is given an important place. Maltby (1926) recommends a number of methods for preserving different types of plant parts *with* colour. For preserving green plant parts, he suggests keeping the specimen in a solution of copper sulphate and bubbling in sulphur dioxide gas. Another suggestion from him is to boil the plant material for a few minutes in a dilute solution of copper acetate (or sulphate) in glacial acetic acid. Both his suggestions require costly equipments, and offer but limited success. Excellent suggestions from Mackenzie (1928) contain a number of formulae comprising of sulphurous acid, formalin, glycerine, copper sulphate, paraffin oil, etc., for preserving different plant parts, with particular attention to fruits. Her formulae vary with the plant parts, and are not suitable for preserving whole plants. Schully (1937) recommends his formula B, requiring the use of copper sulphate, sulphuric acid and sodium sulphite, for retention of the yellow colour of flowers and the green colour of other plant parts. The cost, non-availability of the same solution for a second use, and the effects on the other colours of the specimens, do not lend support to his method. Blaydes (1937) suggests storage of specimens in a ·2 % solution of copper sulphate in the standard formalin-acete-alcohol. Johansen (1940) substitutes propionic acid for acetic acid in that solution. Their solution is unsatisfactory, since it is discoloured by the sulphate. Johansen (1940) also enumerates Conant's Hot Method, Conant's Cold Pack Method and Keefe's Preserving Fluid, for preserving green plant parts *with* form and colour. They require acetic acid, copper acetate, formalin, alcohol, etc. which increase the cost enormously. He suggests also an easy and simple way of keeping the plant materials in a solution of boric acid in glycerine. In this solution the colour of the material is however, lost after a few months.

It is gathered from the above-furnished information, that copper salts play an important role in the preservation of green plant materials *with* colour. Hard, blue crystals of copper sulphate are therefore preferred for this work. The salt has to be dissolved in a liquid that will penetrate the plant tissue. Formalin serves this purpose well.

Method

A 10 % solution of formalin in water, is prepared. Finely-powdered copper sulphate is dropped little by little at prolonged

intervals into the formalin solution, till a large quantity of the salt accumulates at the bottom without dissolving any more in the solution at room temperature.

A freshly-collected whole plant of *Scilla indica* Baker is kept immersed in the solution of copper sulphate, mentioned above. The container is sealed and put by for seven days. Then the specimen is transferred to a 5 % formalin solution (without copper sulphate). This is the final preservation fluid. Sometimes it turns slightly bluish green after a few months have lapsed. All that is necessary then, is to renew the fluid. After a couple of renewals, the solution remains colourless. A specimen treated this way, has retained its true colours for over ten years.

Other plant materials subjected to this treatment, using the first-prepared saturated solution of copper sulphate in 10 % formalin as the *stock solution,* are *Allium cepa* Linn., *Raphanus sativus* Linn., *Solanum tuberosum* Linn. *Pongamia pinnata* Merr., *Vitis vinifera* Linn., *Bulbophyllum* sp., and *Pectis papposa.* All these specimens have also shown excellent retention of colour for over six years. No deterioration has been noticed both in regard to the colour of the non-chlorophyll-bearing tissues, and in regard to the original turgidity of the specimens.

Numerous experiments were conducted, varying the concentration of the solutions, and also varying the duration of the treatment in the copper sulphate solution. The schedule described above, was however found to be the most successful one.

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REFERENCES

Bishop, A. H. (1932) Treatment of Foliage for Museum Exhibition.. *Mus. J., Lond.,* 31: 483.

Blaydes, G. W. (1937) Preserving the natural colour of plants. *Science,* 85: 126-127. (Original not studied. Source: Johansen, 1940).

Butler, O. (1918) On the Preservation of Phytopathological Specimens in their Natural Colours. *Phytopathology*, 8: 66-68. (Original not studied. Source: Maltby, 1926).

Cleghorn, H. F. C. 1838) *Directions for Collecting and Preserving Botanical Specimens*, pp. 14, Oriental Press, Madras.

Johansen, D. A. (1940) *Plant Microtechnique*, pp. 523, McGraw-Hill Book Company, Inc., New York and London.

Mackenzie, A. D. (1928) The Preservation Of Fruit In Natural Colours. *Mus. J., Lond.* 28: 6-16.

Maltby, F. E. (1926) Improved Methods of Preserving Botanical Specimens in their Natural Colours. *Mus. J., Lond.* 25: 329-332.

Matthews, J. M. (1920) *The Application of Dyestuffs*, John Wiley and Sons, Inc., London and New York (Original not studied. Source: Rowley, 1943).

Rowley, J. *Taxidermy and Museum Exhibition*, pp. 342, D. Appleton Century Co., Inc., New York and London.

Scully, F. J. (1937) Preservation of Plant Material in Natural Colours. *Rhodora*, 39: 16-19.

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